MP MASTER
MA32846

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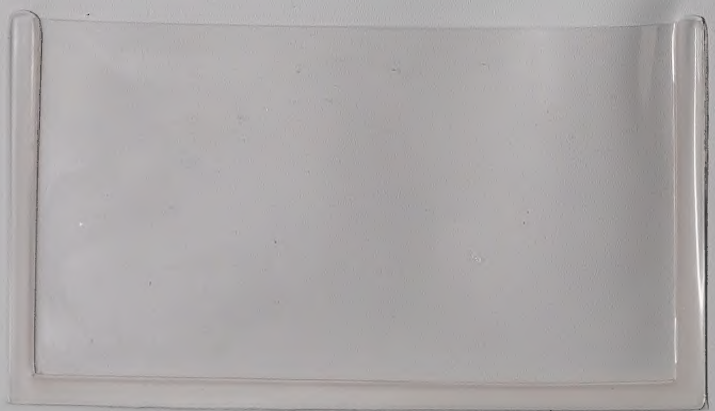

DIONEX

Steve Albright

AI-450 CHROMATOGRAPHY SOFTWARE USER'S GUIDE

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About This User's Guide

This manual covers the Dionex AI-450 Chromatography Software Program Release 3.30. This manual assumes you already understand concepts unique to the Microsoft Windows™ Operating Environment. If you do not know how to use programs that run under Windows, review the Windows documentation before trying to use the AI-450 Chromatography Software.

This manual is a reference guide and provides a complete description of each feature of the AI-450 Chromatography Software. The manual consists of the following chapters and appendixes:

- *Setting Up Your System* provides the information you need to install the hardware and the AI-450 software. It is very important that you carefully read and follow this information before you begin using the AI-450. If you must install the AI-450 software without reading the manual, insert the **Master Release Disk #1** in the **A** drive, type **A:SETUP450** and then press the **<ENTER>** key.
- *Getting Acquainted* provides a brief overview of the Dionex AI-450 and a brief description of individual programs.
- *Menus* provides an overview of the AI-450 program menus and how to use them.
- The chapters that follow *Menus* provide detailed descriptions of the AI-450 programs. Each chapter is named after the program it discusses.
- The appendixes contain other useful information, including a glossary, detailed descriptions of the peak integration and quantitation calculations done by the AI-450 software, troubleshooting advice for the hardware installation, and a brief guide to using DOS.

Cross-references in this manual are in the following format: *Chapter Name/Chapter Section/Chapter Subsection* (e.g., *Setting Up Your System/System Requirements* or *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Report*).

In the manual, as well as throughout the AI-450 software, the major components of your Dionex Chromatography System are referred to by abbreviations; see the table below for some examples.

Abbreviation	Module
AI-450	AI-450 Data System
ACI	Advanced Computer Interface
AGP	Advanced Gradient Pump
ARC	AutoIon Reagent Pump and Controller
ASM	Automated Sampler
CDM	Conductivity Detector Module
CES	Capillary Electrophoresis System
CHA	Advanced High-Pressure Chromatography Module
GPM	Gradient Pump Module
PAD	Pulsed Amperometric Detector
PED	Pulsed Electrochemical Detector
UDM	UV-Visible Detector Module
VDM	Variable Wavelength Detector Module

Release Notes

Any important information that was not available at the time this manual was printed is contained in the AI-450 on-line Help and in the README.TXT file on AI-450 Disk #1. During installation, the SETUP450 program automatically copies README.TXT to the directory you choose for your AI-450 files. You can read this information by using one of the methods listed below.

If the AI-450 software has not yet been installed on the computer, use the following procedure:

1. Insert AI-450 Disk #1 into the A: drive.
2. With a DOS prompt on the screen, enter TYPE A: README.TXT¦MORE.
3. Read the text on the screen; advance the text one page at a time by pressing any key.
4. You can print a copy of the text by entering TYPE A:README.TXT > PRN. If this is inconvenient, the README.TXT file can be printed from most text editors or word processing software, including Windows Notepad, Windows Write, Microsoft Word®, WordPerfect®, and WordStar®.

   NOTE: DO NOT SAVE ANY CHANGES TO THE FILE.

If the AI-450 software has been installed on the computer, the most convenient way to read the release notes is by using the on-line Help feature. For more information about the use of Help, refer to *Menus/The Help Menu*.

1. First, select the Index... command from the Help menu of any AI-450 program. The Help index screen for the program will appear.
2. Note the series of buttons immediately below the menu bar of the Help index screen. Click on the button labeled Contents (or Index) and the AI-450 Help Main Index will appear.

   NOTE: The button label depends on which version of Windows is installed on your computer; it is "Contents" in Windows 3.1 and "Index" in Windows 3.0.
3. Click on AI-450 Release Notes to display the AI-450 Release Notes Index. Click on any underlined topic to view more information.

NOTE: If you purchased your computer from Dionex, all necessary software and hardware will be installed for you. These instructions are intended for reference only.

Installing the AI-450 Data System is a two-part process.

- First, the correct hardware must be installed and connected to your computer. If you are installing the AI-450 for the first time or if you are upgrading your system, refer to the hardware installation instructions in this chapter. Consult the *Advanced Computer Interface Operator's Manual* (Doc. No. 034011) and the manuals for your computer and its accessories for additional information.
- Second, the AI-450 Chromatography Software must be installed and configured. Before using the software for the first time, there are a few setup procedures you **must** follow to ensure that the software works properly. Please carefully read and follow the information in this chapter before using the AI-450 software.

System Requirements

Your computer system must include the hardware and software listed below in order to run the AI-450 Chromatography Software.

Hardware Requirements

Computer

• **Computer**	IBM PC/AT, IBM PS/2 Model 50 through 80, or 100% compatible computer
• **RAM Memory**	2 Mbytes minimum; 4 Mbytes or more recommended
• **Fixed Disk Drive**	40 Mbytes minimum; 80 Mbytes or more recommended. The drive must be formatted and configured as drive C:.
• **Floppy Disk Drive**	5.25-in. High Density (1.2 Mbytes) or 3.5-in. High Density (1.44 MBytes). The drive must be configured as A:.
• **Disk Controller**	Any controller compatible with your computer and disk drives
• **Serial Ports**	One for each Dionex Advanced Computer Interface, Spectra-Physics SP8880 or AS3500 Autosampler, and Isco Foxy II Fraction Collector (except with AI-450 Data System Model 3)
• **Display Adapter**	Windows-compatible VGA (Video Graphics Array) or EGA (Enhanced Graphics Adapter) port
• **Display**	VGA or EGA monitor compatible with your computer's display adapter
• **Keyboard**	Any keyboard compatible with your computer

Peripherals

- **Mouse** — Windows-compatible mouse (bus-style preferred)
- **Printer** — Windows-compatible printer suited to your needs (parallel-type recommended)
- **Printer Cable** — Cable designed to interface your printer and computer

Software Requirements

- MS-DOS or PC-DOS Operating System Version 3.3 (or higher) installed on the hard disk
- Microsoft Windows Version 3.0 or higher

Installing the AI-450 Hardware

Installing the Computer

Before beginning, read the installation manuals for your computer, mouse, and printer. If you have an AI-450 Model 3 Data System, also read the manual for the GPIB interface board.

You need to know how to turn on the computer, which disk drive is drive A:, and which keys reset, or boot, the computer.

Installing the Computer Accessories

Before installing the Windows Operating Environment and the AI-450 Chromatography Software, verify that the following accessories have been installed in your computer and that everything operates correctly.

The hard disk is installed, formatted, and designated as drive C:. The following directories will have already been created and the appropriate files copied into them:

Directory Name	Files
\DOS	DOS operating system and utilities
\WINDOWS	Windows Operating Environment
User-selected directory	AI-450 Chromatography Software
\CONFIG *	ACI Configuration files
\DATA *	AI-450 raw data files and result data files
\METHOD *	AI-450 Method files, including Control Timed Event files (*.TE) and Gradient Programs (*.GPM)
\SCHEDULE *	AI-450 Schedule files

* Subdirectories under the user-selected directory for AI-450 software

If your computer was purchased from and/or installed by Dionex, the GPIB interface board (for the AI-450 Model 3 Data System only), an enhanced graphics adapter, and the mouse interface are installed and tested at the factory.

If you purchased the printer from Dionex, the printer interface will also be installed and tested for you.

Installing the GPIB Interface Board (Relay-Only Model 3 Data System)

The Model 3 Data System requires a GPIB (IEEE-488) interface board to communicate with the Dionex Advanced Computer Interfaces (ACIs). This board is available from Dionex or may be purchased directly from National Instruments (Model PC/2A for PC/AT compatible computers or Model MC-GPIB for PS/2 computers).

Before you install the AI-450 software, install the GPIB interface board in one of the expansion slots in your computer as directed in the instructions that accompanied the board. Do not change any of the default switch settings on the board.

Connecting the AI-450 to the Chromatograph

Before installing your system, review the *Advanced Computer Interface Operator's Manual* (Doc. No. 034011). This manual provides detailed instructions for installing the Advanced Computer Interface (ACI) and making all of the connections between the ACI and the chromatograph and its accessories.

WARNING: Make sure that the POWER switches on the ACI, the computer, and all of the system components are turned off before you begin making the connections to the ACI and the computer.

Parallel Interface Connections

The ACI uses a 50-line parallel interface for Parallel Remote control of suitably equipped Dionex modules. A single-channel ACI can control up to four modules via this interface. A dual-channel ACI can provide control of an additional four modules. Use 50-line ribbon cables to connect the ACI to the modules in the system.

NOTE: The relay-only Data Systems do not include parallel interface control.

Relay and TTL Connections

The four RELAY and four TTL connectors on the rear panel of the ACI provide control of chromatography accessories that require a contact closure or digital TTL logic levels (e.g., a stream-select valve). These connections are assigned to either System 1 or System 2, using the Configuration Editor program. Use relay cables (P/N 041558) to connect Dionex accessories that use telephone- style connectors. Other cables are available for other types of relay/TTL connections.

Switched AC Outlets

The AC outlets on the rear panel of the ACI provide simple on/off control of additional accessories (e.g., recorders and sampling pumps). The total current drawn by each AC outlet must not exceed 2 amps. The AC outlets are assigned to either System 1 or System 2, using the AI-450 Configuration Editor program. Connect the line cord of each accessory to an AC outlet on the ACI rear panel.

NOTE: The Relay-Only Model 1 Data System does not include the switched AC outlets.

Connections from Analog Detectors to the ACI

Each channel of the ACI can collect data from up to two analog detectors. Use coaxial cables (P/N 035883) to connect the recorder output of your detector to the DET 1 and DET 2 connectors on the ACI rear panel.

NOTE: The AI-450 software supports up to two detectors per system.

Connecting the Advanced Computer Interface to the Computer

Setting the ACI Device Address (AI-450 Model 3 Data System Only)

For the first Advanced Computer Interface (ACI), use the factory-set default address of 5. If you are using additional ACIs, use settings 6 through 10, successively. Instructions for setting the device address are given in Appendix B of the *Advanced Computer Interface Operator's Manual* (Doc. No. 034011).

Setting the Baud Rate (AI-450 Data System Models 1 and 2)

The AI-450 software supports RS-232 operation at 9600 baud (RS- 232 Baud Rate switch position 3 on the ACI rear panel). See Appendix B of the *Advanced Computer Interface Operator's Manual* (Doc. No. 034011) for complete instructions.

Connecting the ACI to the Computer

Using the GPIB or RS-232C cable provided, connect the connector on the rear panel of the Advanced Computer Interface (ACI) to the corresponding connector on your computer. Each RS-232 ACI requires a separate RS-232 port. DOS allows up to four serial ports to be installed (COM1 through COM4), but only two ports can be used at one time.

Installing the AI-450 Software

Before installing the AI-450 Chromatography Software on your computer, do the following:

- Create a C: \DOS directory on your hard disk and copy **all** of the DOS utilities into it.
- If you are upgrading from a previous version of the AI- 450 software, print your existing system configuration; use the Print command from the File menu in the Configuration Editor program.

The SETUP450.EXE file included on the AI-450 Master Release disk(s) performs a three-step installation process automatically. **SETUP450 can only be run from the DOS system level prompt (C:). If you are running Windows, return to DOS by double-clicking on the System box in the upper left corner of the Program Manager window.**

- First, SETUP450 checks to make sure that Windows is installed. If it is not, you will be instructed to install Windows and then rerun SETUP450.
- Next, SETUP450 creates a series of subdirectories in which the files used by the AI-450 software are stored. The programs which comprise the AI-450 software are copied into the \DX directory.

- Finally, SETUP450 modifies the AUTOEXEC.BAT file so that whenever the computer is booted Windows is loaded automatically and the AI-450 MainMenu is displayed. The CONFIG.SYS file is also modified to provide optimum performance of the AI-450 software.

Installation Procedure

1. Turn on the computer.
2. Insert the AI-450 Master Release disk #1 into drive A:.
3. At the DOS system prompt C:, type **A:SETUP450** and press [Enter].
4. The SETUP450 program will guide you through the steps required to copy the AI-450 programs onto your hard disk. Follow the step-by-step instructions as they appear on the screen.
5. After the AI-450 software is installed on your hard disk, SETUP450 will add the following lines to CONFIG.SYS, the system configuration file:

   ```
   FILES=30
   BUFFERS=20
   ```

6. SETUP450 modifies the AUTOEXEC.BAT file by adding the paths selected by the user for Dionex programs and windows. The original AUTOEXEC.BAT file is renamed AUTOEXEC.USR. In addition, SETUP450 adds the following lines to the AUTOEXEC.BAT file:

   ```
   SET TEMP=C:\TEMP
   DEL C:\TEMP\~*.TMP
   WIN MAINMENU
   ```

   After installation is complete, this file can be edited to add additional commands (e.g., load a mouse driver).
7. After running SETUP450, store your original Windows disks and the original AI-450 disks in a safe place away from magnets. If the installed copy of either software package is ever damaged, you will need to rerun SETUP450.

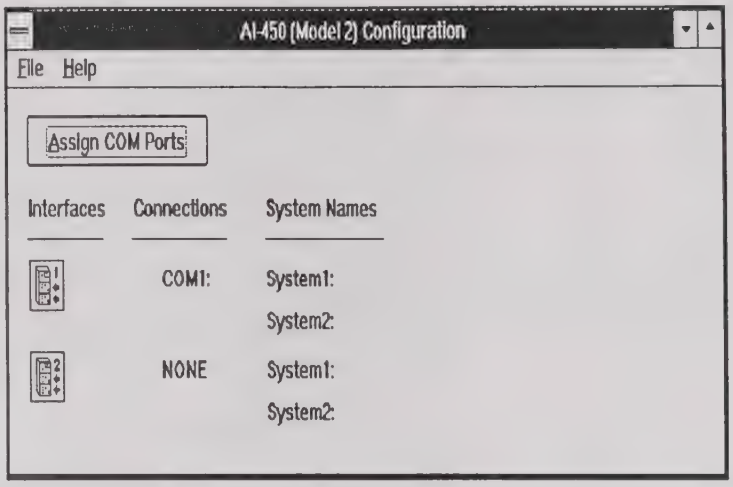
Configuring Your System

The Advanced Computer Interface (ACI) functions as the communications and control link between your computer and Dionex chromatography modules. After installing the software and hardware, use the Configuration Editor program to tell the AI-450 how your system components are connected to the ACI. For Data System Models 1, 1R, and 2, you must also indicate to which computer communication (COM) port each interface is connected.

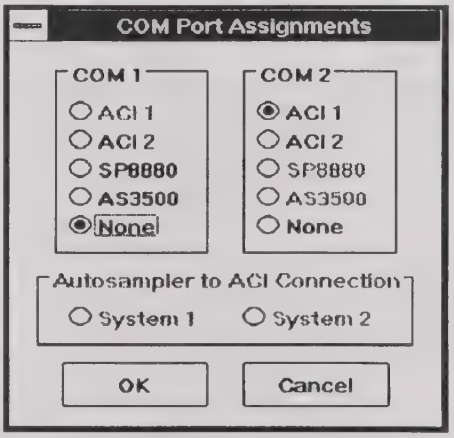
NOTE: For a detailed description of the Configuration Editor program, refer to the *Configuration Editor* chapter later in this manual.

Configuration Procedure

1. Double-click on the Configuration icon in the AI-450 MainMenu. The Configuration Editor main window will appear:

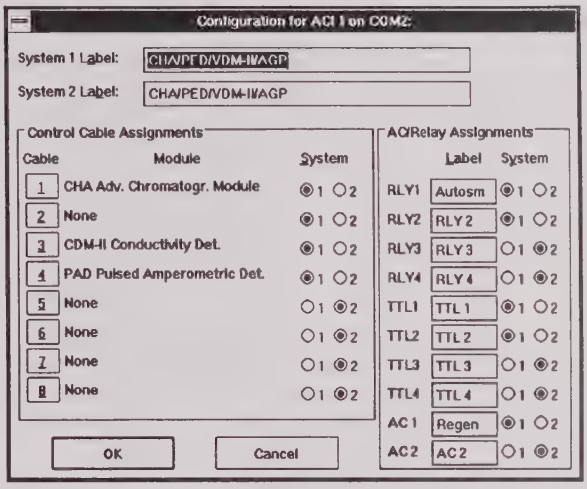


2. Click the mouse cursor on the Assign COM Ports command button to open the COM Port Assignments dialog box. Here is the dialog box for Model 1:



3. If you have a Data System Model 1, 1R, or 2 (RS-232), click on the Assign COM Ports button to specify to which computer communication port each ACI is connected. Consult your computer manual if you are uncertain about the port numbering.

4. To check the configuration of an ACI, click on the corresponding icon button in the Configuration Editor main window. When the current configuration is displayed, make sure that it matches the connections you made to the ACI. You may also enter a description of each system in the text boxes at the top of the dialog box.



5. If you need to change an ACI configuration, click on the button corresponding to that cable. A list box will appear naming all of the modules that can be connected to the ACI. Click on the desired module, then click on OK.

   NOTE: If you are using a relay-only Data System model, the buttons corresponding to the cables are grayed out.

6. You must also designate to which channel (Sys 1 or Sys 2) each of the contact-closure relays, TTL control lines, and AC outlets is assigned. These connections are used to control various accessories and, in a dual-channel system, can be allocated as desired between the two channels (Sys 1 and Sys 2).

7. When all ACI configurations are correct, click on OK to save them on disk under the file name CONFIGx.ACI, where x is the number of the ACI. Configuration Editor files are stored in the \DX\CONFIG directory.

8. Terminate the Configuration Editor program by selecting the Close command from the System menu. This will return you to the \DX directory.

What Is the Dionex AI-450?

The Dionex AI-450 Chromatography Software controls chromatographs (LCs, GCs, ICs, SFCs, etc.); automatically collects, processes and reports data; and provides utilities that simplify interpretation of results. The AI-450 programs run under the Microsoft Windows Operating Environment.

The AI-450 software runs on a workstation consisting of an IBM-compatible personal computer equipped with a mouse and a printer, plus one or more Dionex Advanced Computer Interfaces (ACIs).

The AI-450 Chromatography Software controls and monitors up to six separate ACIs. Each ACI, in turn, controls one dual-system chromatograph and collects and stores data from up to two detectors per system. The ACI has two functions: 1) to control the chromatograph and any accessories (e.g., autosamplers and sampling pumps) and 2) to collect and store data from the detectors until the computer is ready to process it.

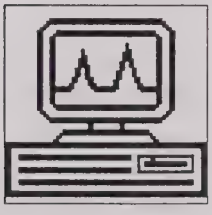
The AI-450 software retrieves raw data from the ACIs, performs data reduction and quantitation calculations, and stores the data on disk. It also provides post-run operations for comparing analyses, changing baseline conditions, and re-integrating data.

Overview of the AI-450 Chromatography Software

The AI-450 software is organized as a set of programs, each with a specific function, which appear both as icons in the AI-450 Program Menu and as files in the \DX directory. Program files are identified by the filename extension ".EXE". Each program in the AI-450 software also creates and/or uses additional files.

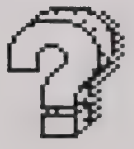
This chapter presents an overview of the programs. For a detailed description of the menus and parameters for each program, refer to the appropriate chapter in this manual.

The AI-450 MainMenu Program



The **AI-450 MainMenu program** (MAINMENU.EXE) is automatically displayed when you turn on or reboot your computer. The menu presents icons of the programs that comprise the AI-450 software package. Clicking on a program icon once displays a brief description of the program's function; double-clicking on the icon starts the program.

The Help Program

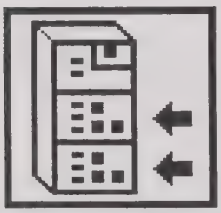


The **Help program** is an on-line facility that provides information about the AI-450 software. The Help program includes brief instructions on how to use each AI-450 program and instructions for carrying out specific tasks. The Help program is typically used while running another program. See *Menus/The Help Menu* for more information.

Installation Functions

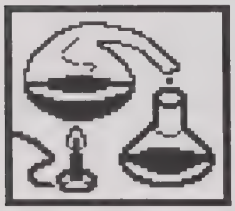
SETUP450.EXE is the DOS-level program you use to install the AI-450 software. It verifies that Windows is installed, then creates a \DX directory on your hard disk and copies the AI-450 Chromatography Software into that directory. It also creates additional directories in which files created and used by the AI-450 software can be stored. The SETUP450 program is also used to update the AI-450 software to the latest version.

Connections



The **Configuration Editor program** (CONFIG.EXE) is used during installation of the AI-450 or when you change instrument configurations. It configures the interface and the software to the analytical system being used.

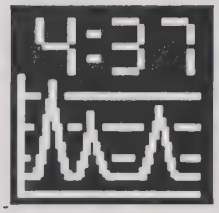
Preparation for Analysis



The **Method Editor program** (METHOD.EXE) is used to create and edit Methods for an analysis or experiment. A Method contains all of the instrument control and data handling instructions needed to run a single analysis.

The **Schedule Editor program** (SCHEDULE.EXE) program is used to create, store, and edit a Schedule of analyses. Each line in a Schedule specifies the sample name, the Method to be used for the analysis, and optional correction factors. Automatic calibrations can easily be incorporated into the Schedule.

Analysis

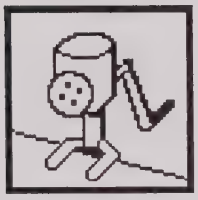


The **Run program** (RUN.EXE) loads a selected Advanced Computer Interface with a Method or a Schedule. Each system's status and real-time chromatogram are displayed separately.

When an analysis is complete, Run directs the computer to retrieve the raw data from the Advanced Computer Interface. It then detects and integrates peaks and calculates component concentrations, based on response factors calculated from calibration standards. Once the calculations are complete, Run stores and/or prints the results, depending on the contents of the Method(s). If Run is connected with any other programs (e.g., Trend or Microsoft Excel) by Dynamic Data Exchange (DDE), it will output results to those programs in the CSV (Comma Separated Variable) format.

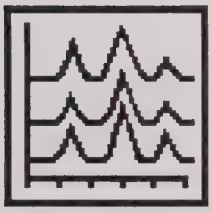
Post-Analysis Data Processing

The **Optimize program** (OPTIMIZE.EXE) allows you to retrieve raw data from the disk drive for additional processing. The data file is first shown exactly as it was processed at the time it was generated. Menu commands allow you to test various treatments of the raw data and update Methods, data files, and report files after making modifications.

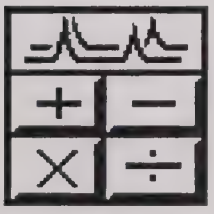


The **Batch program** (BATCH.EXE) allows you to reprocess previously stored data files in a group instead of individually. Batch uses information from Schedules to retrieve the names of the data files to be reprocessed and the Methods to be used. It then stores the reprocessed data as new files and generates new reports.

The **CalPlot program** (CALPLOT.EXE) displays and prints the response vs. amount calibration, the residuals plot, or both, for each component in a multilevel calibration.



The **View program** (VIEW.EXE)allows you to visually compare up to eight stored chromatograms. The chromatograms can be plotted on separate axes or overlaid on a single set of axes.



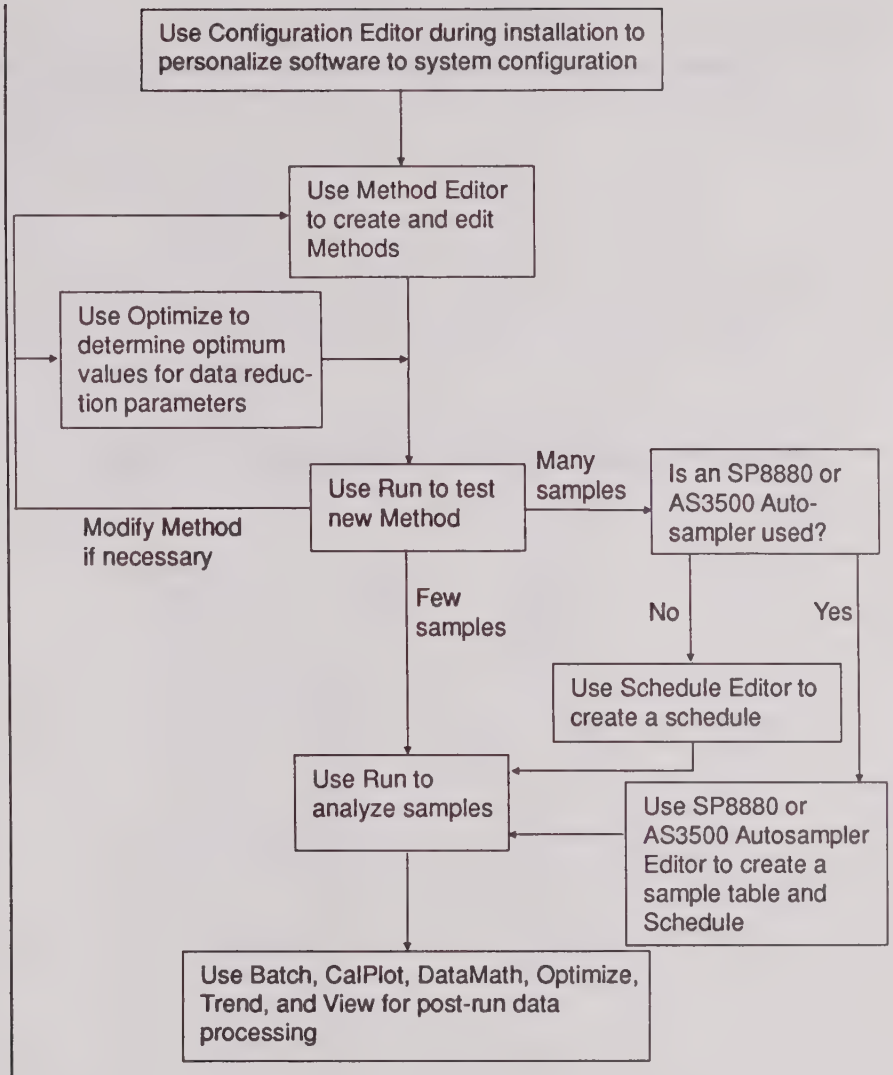
The **DataMath program** (DATAMATH.EXE) calculates and plots the mathematical sum, difference, or ratio of any two raw data files that were collected and stored by the Run program. DataMath can also plot the first and second derivatives of a data file. Calculation results can be stored as a new data file and processed in the same way as any other data file.

Using the AI-450 Software

Once the AI-450 installation is complete and you are familiar with Windows, you are ready to use the AI-450 Chromatography Software.

Before beginning, take a moment to think about the sequence of events that typically takes place as you prepare to do a series of analyses:

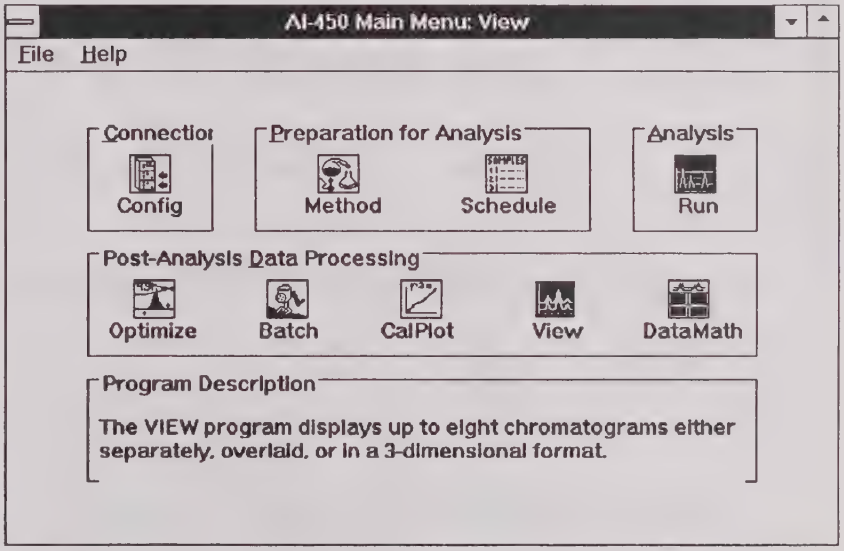
1. Configure the AI-450 to your instrument, using the Configuration Editor program. Unless you change your system configuration, it is only necessary to do this once.
2. Develop one or more Methods or procedures for analyzing your samples, using the Method Editor program.
3. Test the Methods by analyzing calibration standards, using the Run program.
4. If necessary, use the Method Editor, CalPlot, or Optimize program to modify the Methods.
5. Set up a Schedule of analyses using these Methods in some logical sequence, using the Schedule Editor program. The Schedule might include Methods to equilibrate the system, calibrate it, analyze samples, and then shut down the system.
6. Carry out the analysis of your samples, either using one Method or a Schedule of Methods, using the Run program.
7. Examine the results of your analysis, manipulate the data, and compare the results with those obtained from other analyses. For this you might reprocess with the Optimize and Batch programs, calculate ratios or differences with the DataMath program, or compare plots with the View program.


Use Configuration Editor during installation to personalize software to system configuration
Use Method Editor to create and edit Methods
Use Optimize to determine optimum values for data reduction parameters
Use Run to test new Method
Many samples
Is an SP8880 or AS3500 Auto-sampler used?
Modify Method if necessary
Few samples
No
Yes
Use Schedule Editor to create a schedule
Use Run to analyze samples
Use SP8880 or AS3500 Autosampler Editor to create a sample table and Schedule
Use Batch, CalPlot, DataMath, Optimize, Trend, and View for post-run data processing

The AI-450 MainMenu Program

MainMenu

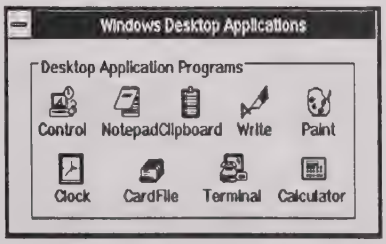
The AI-450 Program Menu (MAINMENU.EXE) displays icons representing all the AI-450 software programs whenever you turn on or reboot your computer. Clicking on a program icon once displays a brief description of the program's function; double-clicking on an icon starts the program. When you exit an AI-450 program, the AI-450 Program Menu is redisplayed.



File Menu

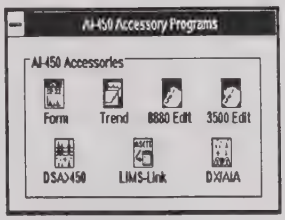
The File menu has four commands.

- Selecting Run Windows Desktop Applications... displays this screen:



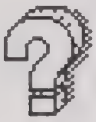
The icons represent various Windows desktop applications programs. Clicking on a program icon once displays a brief description of the program's function; double-clicking on an icon starts the program.

- Selecting Run AI-450 Accessory Programs... displays a screen containing icons for the optional AI-450 programs. Clicking on a program icon once displays a brief description of the program's function; double-clicking on an icon starts the program.



- Selecting the Confirm Exit command gives you the choice of being asked to confirm, via a message box, your desire to exit the MainMenu program before the program is closed.
- Selecting the Exit AI-450 command closes the MainMenu program.

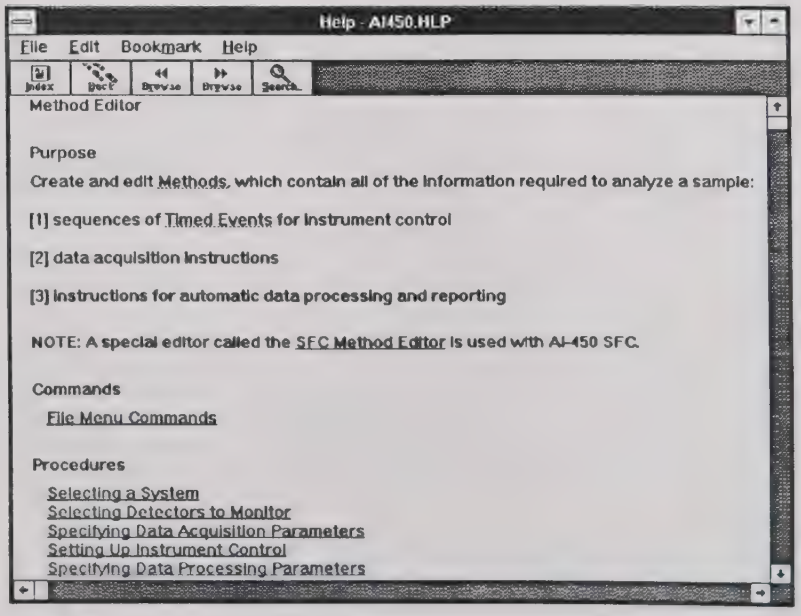
The Help Menu



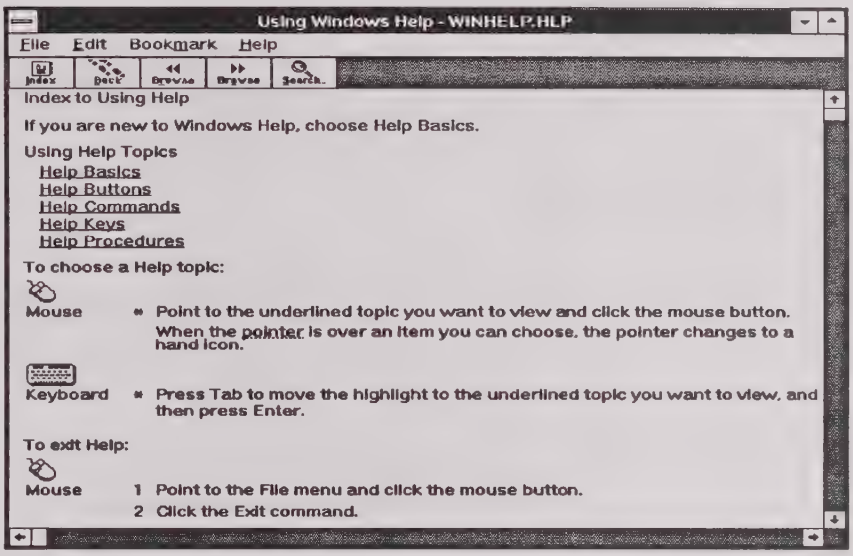
Each of the AI-450 programs contains a Help menu to provide quick answers to your questions about the program. The Help menu lists just two commands:

- Selecting About the program... displays the software release level and copyright date.
- Selecting Index... displays a listing of the purposes of the program currently running, an index of commands and procedures, and various related topics. For example, the initial index screen for the Method Editor program is shown below. Note that some of the topics in the index are underlined; to see more information, click the mouse on the underlined text.

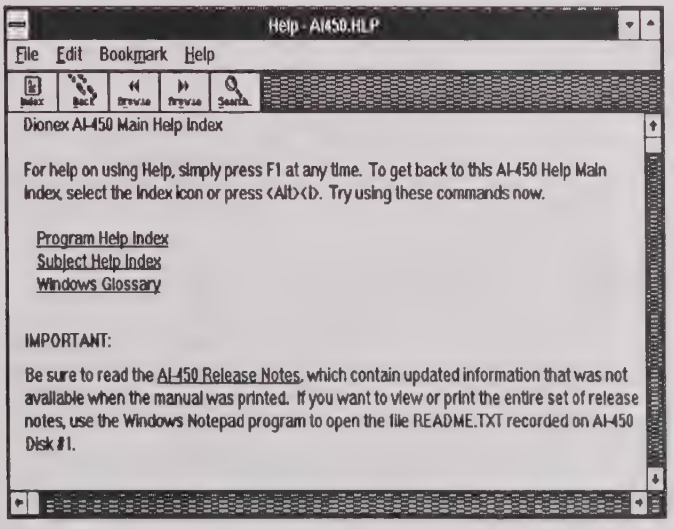
  NOTE: The text on your index screens may differ slightly from that shown here, depending on whether Windows 3.0 or 3.1 was installed on your computer.


Help - AI450.HLP
File Edit Bookmark Help
Index
Back
Browse
Browse
Search
Method Editor
Purpose
Create and edit Methods, which contain all of the information required to analyze a sample:
[1] sequences of Timed Events for instrument control
[2] data acquisition instructions
[3] instructions for automatic data processing and reporting
NOTE: A special editor called the SFC Method Editor is used with AI-450 SFC.
Commands
File Menu Commands
Procedures
Selecting a System
Selecting Detectors to Monitor
Specifying Data Acquisition Parameters
Setting Up Instrument Control
Specifying Data Processing Parameters

If you need extra assistance to use a program index screen, press the F1 function key to access complete information about Help. The initial Help screen is shown below. As with all Help screens, you can use the scroll bar at the right side to view additional information. To return to the program index screen, click the cursor on the Back button below the menu bar.

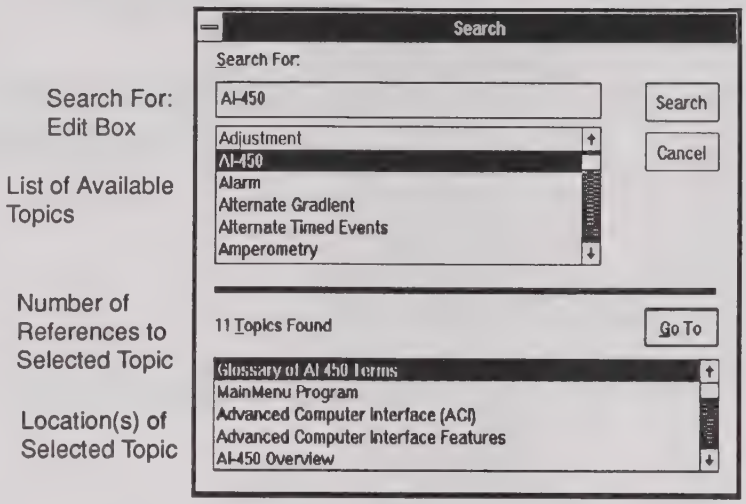

Using Windows Help - WINHELP.HLP
File Edit Bookmark Help
Index
Back
Browse
Browse
Search
Index to Using Help
If you are new to Windows Help, choose Help Basics.
Using Help Topics
Help Basics
Help Buttons
Help Commands
Help Keys
Help Procedures
To choose a Help topic:
Mouse
• Point to the underlined topic you want to view and click the mouse button.
When the pointer is over an item you can choose, the pointer changes to a hand icon.
Keyboard
• Press Tab to move the highlight to the underlined topic you want to view, and then press Enter.
To exit Help:
Mouse
1 Point to the File menu and click the mouse button.
2 Click the Exit command.

Or, if you have a question about some aspect of the AI-450, you can switch from the program index screen to the overall AI-450 Help Index by clicking the mouse cursor on the Index button below the menu bar. To view more information about any of the topics presented in the AI-450 Help Index, click on the underlined text. Here is the initial screen of the index:


Help - AI450.HLP
File Edit Bookmark Help
Index
Back
Browse
Browse
Search
Dionex AI-450 Main Help Index
For help on using Help, simply press F1 at any time. To get back to this AI-450 Help Main Index, select the Index icon or press <Alt><D. Try using these commands now.
Program Help Index
Subject Help Index
Windows Glossary
IMPORTANT:
Be sure to read the AI-450 Release Notes, which contain updated information that was not available when the manual was printed. If you want to view or print the entire set of release notes, use the Windows Notepad program to open the file README.TXT recorded on AI-450 Disk #1.

After selecting any of the options presented here, you can return to the previous screen by clicking the cursor once on the Back button below the menu bar. Clicking on the Back button repeatedly allows you to backtrack through an entire sequence of topics.

The search function enables you to quickly locate information anywhere in the Help program. First, click on the Search button below the menu bar to display the Search dialog box:



Use one of these methods to select a topic:

- Type the name of the topic, or just the first few letters of it, in the Search For: edit box. As you type, the software will automatically scroll through the alphabetical list of available topics until it finds the selected topic, or the closest match. The selected topic will be highlighted.
- Use the scroll bar beside the list of available topics to view the options. When you locate the desired topic, click on the name to highlight it.

Click on the Search command button to initiate a search for the selected topic. The number of occurrences of the selected topic, as well as their locations, will be displayed in the dialog box. Click first on any location and then on the Go To command button to display the corresponding Help screen.

To exit Help and return to the program currently running, click the mouse cursor on the File command at the top of the screen, and then click on Exit. You can also exit Help by keying the letters <F> and then <X> while holding down the <Alt> key.

The File Menu

A File menu, a standard element of most Windows applications, also appears in each of the AI-450 programs. File menu commands typically include New, Open, and Save As. These commands act on entire files.

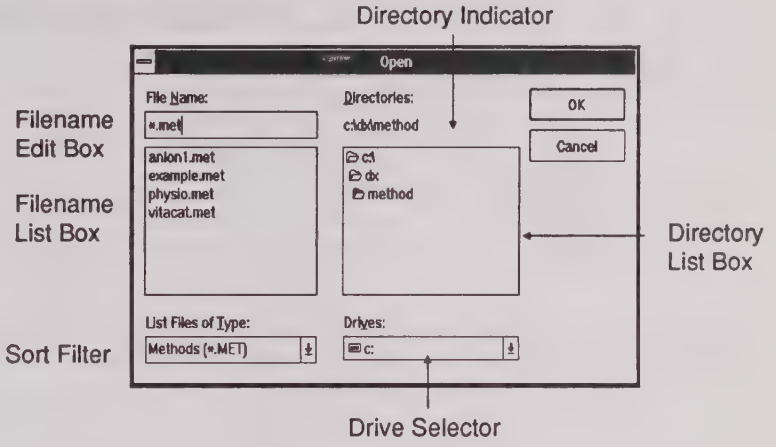
A file may contain a system configuration (.ACI), a Method (.MET), a Schedule (.SCH), or data (.Dxx or .Rxx), where xx is a number from 01 to 99.

New

New clears any files from memory and resets all parameters to their default values. Use this command to create new files.

Open...

Open... retrieves a file from disk and stores it in computer memory. Other commands can then be used to view and/or modify it.



The **Filename Edit Box** displays the selected filename. Type the filename directly into the edit box or click on the desired filename in the filename list box to display the name in the edit box.

The **Filename List Box** displays the names of all files stored in the currently selected directory. To select a file from the list box, click on it once and the name will appear in the filename edit box. Double-clicking on a filename selects it and also opens the file.

The **Directory Indicator** shows the full pathname for the current directory. This path lists nested directories which lead to the current directory.

The **Directory List Box** displays the directories on the current drive. Double-click on a directory to display its files in the filename list box. If the file you want is not in this directory, change the directory.

The **Drive Selector** lets you change the selected disk drive. Click on the arrow to open the drop-down box, then use the scroll bar at the right side to view all the drive options. Double-click on a drive to select it.

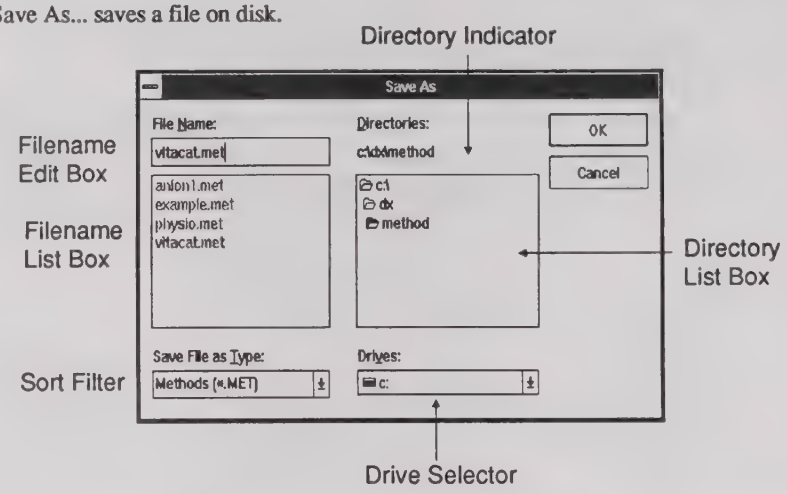
The **Sort Filter** lets you limit the files displayed in the filename list box to those files that match certain criteria. Click on the arrow to open the drop-down box and display a list of available sort filters; double-click on a sort filter to select it.

OK retrieves the specified file from disk and displays the information in the file. Pressing [Enter] has the same effect as clicking on OK.

Cancel closes the Open dialog box without taking any action.

Save As...

Save As... saves a file on disk.



The **Filename Edit Box** displays the selected filename. Type the filename directly into the edit box or click on the desired filename in the filename list box to display the name in the edit box.

The **Filename List Box** displays the names of all files stored in the current directory. To select a file from the list box, click on it once and the name will appear in the filename edit box. Double-clicking on a filename selects it and also opens the file.

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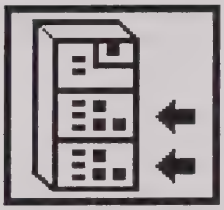
The **Drive Selector** lets you change the selected disk drive. Click on the arrow to open the drop-down box, then use the scroll bar at the right side to view all the drive options. Double-click on a drive to select it.

The **Sort Filter** lets you limit the files displayed in the filename list box to those files that match certain criteria. Click on the arrow to open the drop-down box and display a list of available sort filters; double-click on a sort filter to select it.

OK stores the specified file in computer memory. Pressing [Enter] has the same effect as clicking on OK.

Cancel closes the Save As dialog box without taking any action.

THE CONFIGURATION EDITOR PROGRAM



The Configuration Editor program specifies how the modules and accessories that comprise your chromatograph are connected to each Advanced Computer Interface (ACI), and how the interface and certain accessories are connected to the computer. The Configuration Editor is used during the initial installation of the AI-450 Data System and whenever a module is added to or removed from the system; see the chapter entitled *Setting Up Your System* for more information.

The AI-450 Chromatography Software can control and monitor up to six separate ACIs (depending on the AI-450 model). Each ACI controls and acquires data from up to two complete chromatography systems and each of these systems can include up to two detectors.

When used with some AI-450 Data Systems, the program can also control a Spectra-Physics SP8880 or AS3500 Autosampler and an ISCO FOXY 200 Fraction Collector. Additional modules and accessories can be controlled using contact-closure relays, TTL logic levels, and switched AC outlets.

AI-450 Data Systems

This chapter describes various configuration procedures for the AI-450. The correct procedure depends on which AI-450 Data System model you have. Here is a summary of the models and their features:

The Model 1 Data System supports 1 chromatography system only. It supports an autosampler, and provides parallel remote control of up to 4 Dionex modules. It has 4 relay controls, 4 TTL controls, and 2 controlled AC outlets.

The Model 1R Data System supports 1 chromatography system only, and provides 4 relay and 4 TTL controls.

The Model 2 Data System supports up to 2 ACIs and up to 4 chromatography systems. Each ACI supports up to 2 systems. The Model 2 can control an autosampler and provide parallel remote control of up to 8 modules, 4 relay controls, 4 TTL controls, and 2 controlled AC outlets per ACI. Each system can have up to 4 parallel modules.

The Model 2R Data System supports up to 2 ACIs and up to 4 chromatography systems. Each ACI supports up to 2 systems. Each system provides a total of 4 relay controls, 4 TTL controls, and 2 controlled AC outlets for allocation among the systems used.

The Model 3 Data System uses a GPIB computer interface to support up to 6 ACIs and up to 12 systems. Each ACI supports 2 systems. The Model 3 can control both an autosampler and a fraction collector, two autosamplers of the same model, **OR** one each of two different autosampler models. The Model 3 provides parallel remote control of up to 8 modules, 4 relay controls, 4 TTL controls, and 2 controlled AC outlets per ACI.

NOTE: When you open the Configuration Editor main window (see the next two pages) for the first time, check the title bar. The Data System model listed there is the one you entered during the AI-450 Installation Program. If the model name in the title bar does not correspond to your Data System model, exit the Configuration Editor. Immediately rerun the Installation Program and correct the Data System model name.

CONFIG.EXE Command Summary

File Menu
Print
Exit

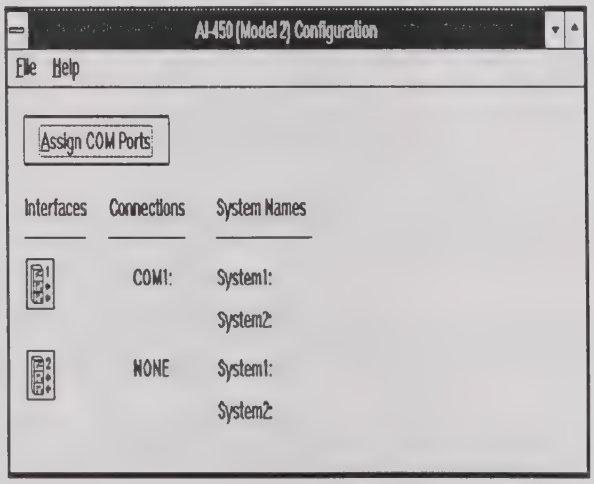
Starting the Configuration Editor Program

To start the Configuration Editor program, double-click on the Configuration icon in the AI-450 MainMenu or on CONFIG.EXE in the directory that holds your AI-450 files. This opens the Configuration Editor main window, which shows the names assigned to each system. Dialog boxes allow you to change each system's configuration and assign functions to the computer communications (COM) ports.

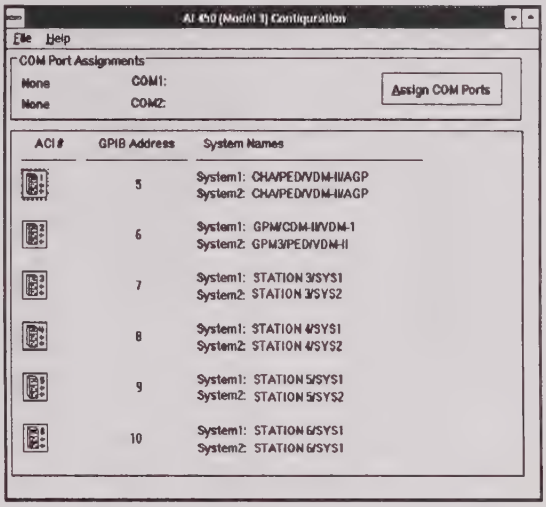
The items shown in the main window and dialog boxes vary, depending on which AI-450 Data System model you have. When an item is grayed out, it is not available for selection.

Configuration Editor Main Window

Main Window - Data System Models 1, 1R, 2, and 2R



Main Window — Data System Model 3

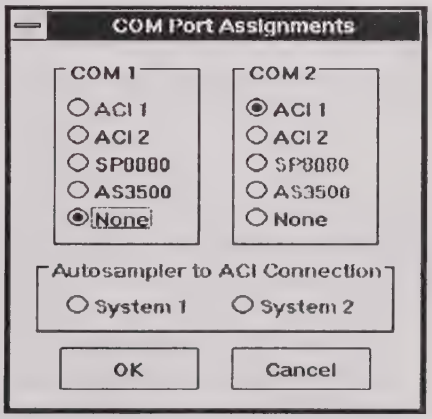

File Help
COM Port Assignments
None COM1:
None COM2:
Assign COM Ports
ACI # GPIB Address System Names
5 System1: CHA/PED/VDM-II/AGP System2: CHA/PED/VDM-II/AGP
6 System1: GPM/CDM-II/VDM-1 System2: GPM3/PED/VDM-II
7 System1: STATION 3/SYS1 System2: STATION 3/SYS2
8 System1: STATION 4/SYS1 System2: STATION 4/SYS2
9 System1: STATION 5/SYS1 System2: STATION 5/SYS2
10 System1: STATION 6/SYS1 System2: STATION 6/SYS1

Communications (COM) Port Assignment

COM Port Assignment — Data System Models 1, 1R, 2, and 2R

If your Data System is a Model 1, 1R, 2, or 2R, you must specify to which of the two computer serial communication (COM) ports each Advanced Computer Interface (ACI) is connected. If you plan to use a Spectra-Physics SP8880 or AS3500 Autosampler, you must also specify the COM port to which the autosampler is connected.

Click the mouse cursor on the Assign COM Port button in the main window to open the COM Port Assignments dialog box. Here is the dialog box for Model 1:

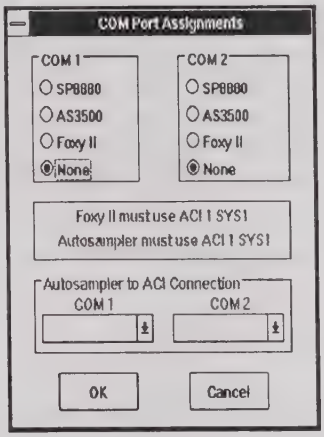


Click your cursor on the buttons in the COM1 and COM2 boxes to make assignments.

NOTE: If you use an SP8880 or AS3500 Autosampler you can have only one ACI, because only two RS-232 COM ports can be used at one time.

COM Port Assignment — Model 3 Data System

Click on the Assign COM Ports button in the Configuration Editor main window to open the COM Port Assignments dialog box for the Model 3 Data System. You do not set the ACI system addresses for the Model 3 because they are preset for the GPIB interface. However, if you use an SP8880 or AS3500 Autosampler or a FOXY 200 fraction collector you must assign each unit to a COM port.



Computer Port Assignment

The fraction collector and autosampler can be assigned to either COM1 or COM2, but they cannot be assigned to the same port.

Fraction Collector Connection

The Foxy II (i.e., the ISCO FOXY 200) can only be used with ACI 1, System 1. When a Foxy II is used, the Model 3 Data System can only control one system.

SP8880/AS3500 to ACI Connection

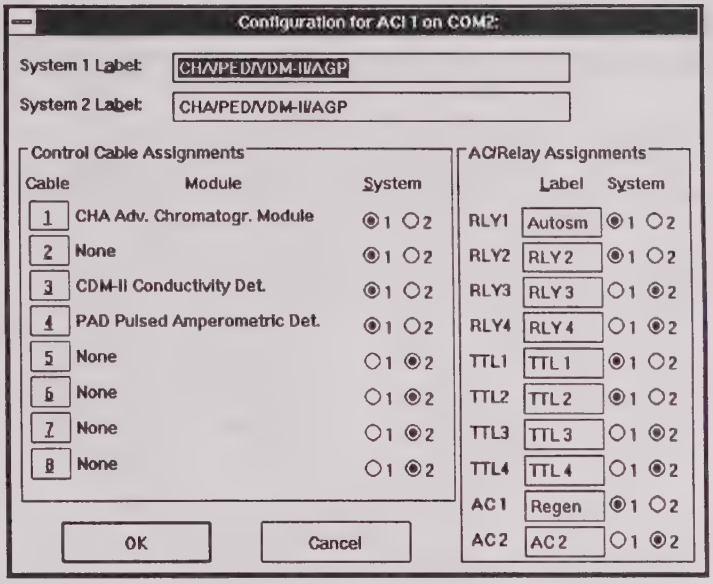
If an SP8880 or AS3500 is used and a Foxy II is **not** used, use one of the drop-down selector boxes under **Autosampler to ACI Connection** to select the ACI and system to which you have the autosampler connected.

If both a Foxy II **and** either an SP8880 or AS3500 are used, the fraction collector and autosampler must always be connected to ACI 1, System 1. When a Foxy II is used, the Model 3 Data System can only control one system.

Advanced Computer Interface (ACI) Configuration

Setting the ACI Configuration

You must configure chromatography systems specifically for each ACI. To begin, click on the icon button in the Configuration Editor main window that corresponds to the ACI you want to configure. A dialog box showing the ACI's current configuration will appear (see next page) or, if no configuration is currently stored, a default configuration will be displayed for reference.



OK stores on disk the ACI configuration currently displayed. The configuration is stored under the filename CONFIGx.ACI, where x is the number of the ACI. The configuration for a given ACI must be saved before any Methods for it can be created.

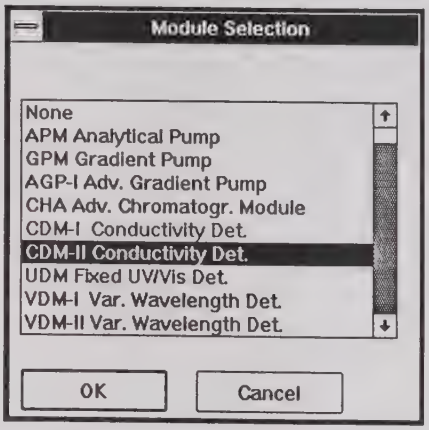
System 1 and System 2 You may create a label of up to 35 characters (including spaces and punctuation) for each chromatograph. The label will be stored in the \CONFIG subdirectory under the filename NOTEx.ACI, where x is the number of the ACI.

Control Cable Assignments (Data System Models 1, 2, and 3 only) Each ACI provides Parallel Remote control of up to two complete chromatographs. A system typically consists of a pump, one-half of a chromatography module, and one or two detectors.

NOTE: Although you can configure up to four detectors for each system of an ACI, only two detectors per ACI can be selected at one time in the Method Editor (see *Method Editor/Data Acquisition Parameters/System*). Also note that the Pulsed Amperometric Detector (PAD) and UV-Visible Detector Module (UDM) output only analog data.

Each system module is connected to the ACI rear panel by a 50-pin ribbon cable. These Parallel Remote cables are numbered from 1 through 8 and correspond to the eight Cable buttons in this screen. The name of the module currently connected to each cable is shown to the right of the Cable button. Make sure that this configuration matches the connections made to the ACI during installation (see *Setting Up Your System/Installing the AI-450 Hardware*).

If a cable assignment is incorrect, click on the button that corresponds to that cable. This opens the Module Selection list box, which lists all the modules that the ACI can control. Select the correct module by clicking on the module name and then on OK.



AC/Relay Assignment(All Data Systems) Each ACI includes four contact-closure relays, four TTL control lines, and two controlled AC outlets for control of additional accessories (e.g., a Dionex Automated Sampler). For a dual-channel interface, these can be allocated as desired between the two systems. A label of up to seven characters (including spaces and punctuation) can be entered for each control.

NOTE: Data System Model 1R has no AC outlet control.

Configuration Files

All configuration files are stored in the C:\DX\CONFIG directory.

- The ACI communication configuration is stored under the filename CONFIG0.ACI.
- The module configuration for each ACI is stored in a file called CONFIGx.ACI, where x is a number from 1 to 6 which corresponds to the ACI being configured.
- The configuration notes you enter for each ACI are stored in a file called NOTEx.ACI, where x is a number from 1 to 6 which corresponds to the ACI being configured.

File Menu

Print

Prints the Configuration file for each Advanced Computer Interface (ACI) controlled by your Data System.

Exit

Closes the Configuration Editor program.

The first step in preparing for an analysis is to develop one or more Methods for analyzing samples. Methods consist of two main elements: data acquisition parameters and data processing parameters. The Method Editor program is used to create, store, and edit Method files containing instructions that tell the AI-450 Data System how to control your chromatograph, how to collect data, and how to process that data and generate a report once an analysis is complete. The Method includes a list of the names, amounts, and retention times of the components in the standard(s) that will be used to calibrate your system.

Default values are supplied for most of the parameters in the Method. To use a default value, simply leave it unchanged; to use a different value, enter the new value in the appropriate space.

Each Method is stored on disk under a unique filename. New Methods can be created by recalling any existing Method, modifying it, and saving the new Method under a different name. Default values usually provide a good starting point when developing a new Method.

As part of a Method, you must enter and store a separate timed events file. This file lists the time-sequenced commands executed by the Advanced Computer Interface (ACI) to control your chromatograph and its accessories. The timed events file is stored under its own filename and the name is stored in the Method file. The timed events file specified in the Method must be stored on disk before the Method can be run.

If your system includes a Dionex gradient pump, you should also create a gradient file to control the pump valves and flow rate. If your system uses a gradient pump and you do not include a gradient file in your Method, you must program the pump from its front panel.

Like the timed events file, the gradient file is stored separately and its name is included in the Method. The gradient file specified in the Method must be stored on disk before the gradient can be used by the Run program.

Because timed events files and gradient files are stored separately, they can be shared by several Methods. This makes editing the control functions of several Methods fast and easy.

NOTE: The most convenient way to edit data processing parameters is through the Optimize program, which lets you work directly with the chromatogram.

Creating Methods for a Dual-Detector System

Each chromatographic system can include up to two detectors. The Method for the first detector controls the operation of the analytical system, as well as the interpretation of the output from the first detector. The output from the second detector is interpreted by a separate Method containing only Integration Parameters, Report Parameters, Calibration, Component Table, and Data Events.

When creating separate Methods for two detectors, keep in mind the following:

- Because the detectors operate in the same chromatographic system, certain parameters must be identical for both Methods. These parameters include the ACI/System specification, the run time, the detector sampling rate, the timed events file, the gradient file, and the PED (Pulsed Electrochemical Detector) file.
- Instrument control parameters for both Methods are stored in the Method file for Detector 1.
- The Data Processing parameters can be different for each Method.
- The filenames for the Detector 1 and Detector 2 Methods are identical except for the extension. The Detector 1 Method uses the extension .MET, while the Detector 2 Method uses the extension .ME2. Extensions are added automatically when the file is saved.

Creating Methods Using a Dionex Gradient Pump

NOTE: For detailed programming instructions for your gradient pump, refer to the *Gradient Pump Operator's Manual* (Doc. 032646) or the *Advanced Gradient Pump Operator's Manual* (Doc. 034463).

Methods created for use with a gradient pump normally include both a gradient file and a timed events file. The gradient file contains a gradient program which controls the flow rate and eluent composition delivered by the pump. If a Method does not include a gradient program, you must remember to program the pump from its front panel. The timed events file contains timed events to control the pump motor (START/STOP) and the gradient clock (RUN/HOLD and RESET).

When setting up timed events for a Method using a gradient pump, keep in mind these points:

- If you are using the valve-switching capability of the gradient pump to control the injection valve, set the pump to RUN at time zero. Make sure that your gradient program includes enough time for loading the injection valve sample loop at the beginning of the program.

  If you are not using the pump to control the injection valve, keep the pump in HOLD (RUN off) at time zero, then set it to RUN when the sample is injected. In either case, it is usually best to run the system under isocratic conditions until the sample is injected.

- When successive samples are analyzed without using a Schedule, it is helpful to include both a RESET and a HOLD (RUN off) command in the last step of the timed events file. Then, if an analysis is ended or aborted, the gradient clock is automatically reset to zero in preparation for the next analysis even if no Method is downloaded between analyses.
- Each time a new gradient file is sent to the gradient pump, the timed events file for the Method must include a toggle from HOLD to RUN (or from STOP to START) for the gradient pump before the new gradient program can control the pump. This is best accomplished by including a HOLD command in the INIT step of the timed events file and a RUN command in the time zero step (see *Run/Considerations When Using a Gradient Pump*).

- The gradient RESET command is a momentary command which resets the gradient clock to time zero. It automatically turns off 0.1 minute after it is turned on. To issue a new RESET command, toggle the command off and then on again. If RUN is on at the time of the reset, the gradient clock continues to run and the gradient program is repeated, starting with the time zero step.

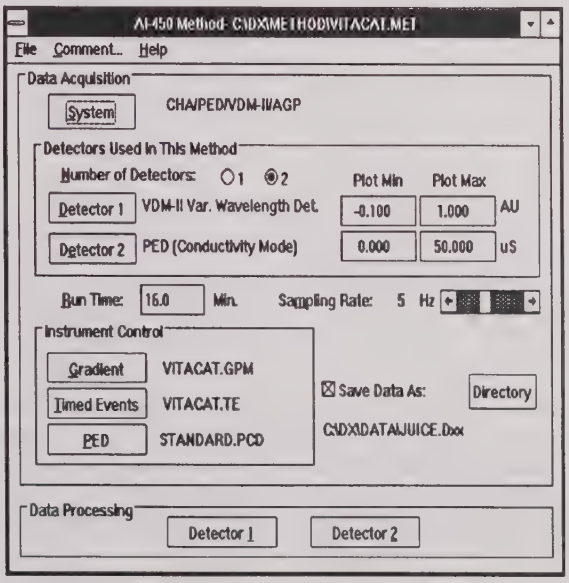
  NOTE: If the last step of the timed events file does not include a RESET command for the gradient pump, the pump will continue operating according to the last step in the gradient program until a new program is downloaded.

After a gradient program is loaded into the gradient pump by the Run program, the new gradient is stored in the gradient pump editing memory and the pump continues to run the current gradient program. When the pump receives an execute toggle (i.e., a HOLD/RUN toggle from the Timed Events file), the program stored in the editing memory is copied into the pump's run memory, where it controls the pump. (A gradient program cannot be run until it is copied into run memory.)

Starting the Method Editor Program

To start the Method Editor program, double-click on the Method icon in the AI-450 MainMenu or on METHOD.EXE in the directory that holds your AI-450 files. The Method Editor main window will appear.

The main window displays the current setting for key Method parameters, and provides command button controls for changing these parameters. There are three pull-down menus. The commands, dialog boxes, and windows associated with each menu are explained on the following pages.



METHOD.EXE Command Summary

- **File Menu**
  - New
  - Open...
  - Save
  - Save As...
  - Print
  - Exit
  - Comment...
- **Data Acquisition Parameters**
  - System
  - Detector Parameters
    - Number of Detectors
    - Detector 1 or Detector 2
      - Detector Assignment List Box
      - Plot Scale
        - Min
        - Max
  - Run Time
  - Sampling Rate
  - Instrument Control Parameters
    - Gradient
      - Gradient Editor Dialog Box
      - Gradient Pump control commands
        - File Menu
        - Edit Menu
    - Timed Events
      - Control Editor
        - Module and RLY-TTL-AC Control commands
        - File Menu
        - Options Menu
    - PED
      - Conductivity Mode
      - DC Amperometry Mode
      - Integrated Amperometry Mode
- **Data Processing Parameters**
  - Detector 1 or Detector 2
    - Data Processing Parameters Dialog Box
      - Integration
        - Peak Detection Parameters
          - Peak Width
          - Peak Threshold
          - Area Reject
        - Reference Peak Parameters
          - Area Reject
      - Data Events
        - Data Events Editor Dialog Box
          - Event Options
          - Current Times and Events

- Calibration
  - Levels
  - Fit Type
  - Force Zero
  - Calibration Update
  - Standardization
  - Response for Unknowns
  - Defaults
    - Sample Volume
    - Dilution Factor
    - Unknown Response Factor
  - Cal. Std. Volume
  - Default Internal Std. Conc. in Samples
  - Sample Units
- Components
  - Component Table Editor
  - Add Components
    - Component Information
    - Component Groups
- Report
  - Text Output
  - Text Sections
    - Components
    - Peaks
  - Report Format
  - Graphics Options
    - Print Chromatogram
  - Peak Labels
    - Peak Number
    - Retention Time
    - Component Name

File Menu

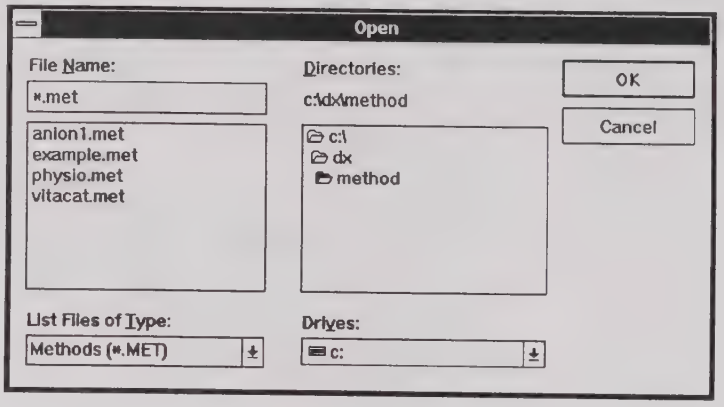
New

Use New when you want to create a new Method. The command clears from memory any Method files already loaded and returns all Settings menu parameters to their default values.

When you select New, all command button controls on the opening screen, except System, are disabled until you specify the system with which the new Method will be associated; see *Method Editor/Data Acquisition Parameters/System* for instructions. This prevents the Method from being used with a system configuration to which it does not apply. Once you specify the System assignment, the remaining command button controls become active.

Open...

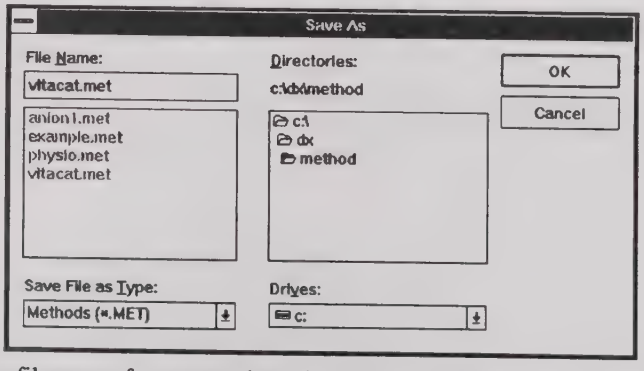
Use this dialog box to select an existing Method file to open:



Save and Save As...

If the Method was previously saved, selecting the Save command rewrites it to disk under the same name as before. If the Method was not previously saved, Save functions in the same way as the Save As... command.

Selecting Save As... opens this dialog box:



Type in a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces) to identify the Method. The filename will be added to the \METHOD directory.

NOTE: If you enter a Method name which is already present in the specified directory, the existing file will be overwritten.

The Method Editor automatically adds the subdirectory \METHOD and the extension ".MET" to the filename but you can change the path, if desired. Refer to Appendix E for information about the use of directories and paths for organizing your files on disk.

If you specify two detectors in the Method, all the data processing parameters may be different. After you save the Method, the parameters for Detector 1 will be saved as the Method name with the file extension .MET. The parameters for Detector 2 will be saved as the Method name with the file extension .ME2.

Print

Prints the Method currently being edited. The next several pages show the printout of a dual-detector Method for the analysis of water-soluble vitamins and inorganic cations in aqueous matrices, using a Dionex PAX-500 column.

DIONEX METHOD PARAMETERS - VITACAT.MET

Method Comment: This method was developed in 1990 for the simultaneous determination of water-soluble vitamins and inorganic cations in aqueous matrices.
Column ID: PAX-500 Serial #1035
Analyst ID: MAR

System Parameters

System Name : CHA/PED/VDM-II/AGP	
Number of Detectors	2
Run Time (minutes)	16.00
Sampling Rate (seconds)	0.20
Detector 1 Type	VDM-2
Detector 1 real time plot scale maximum (AU)	1.000
minimum	-0.100
Detector 2 Type	PED-Cond.
Detector 2 real time plot scale maximum (uS)	50.000
minimum	0.000
Save Data File	Yes
Data File Name: C:\DX\DATA\JUICE.Dxx	

-- DETECTOR 1 PARAMETERS --

Report Options

Create ASCII Report File	No
Print Report	Yes
Print All Components	Yes
Print Components Found	No
Print Missing Components	No
Print All Peaks	No
Print Unknown Peaks	Yes
Print Chromatogram	Yes
Autoscale Chromatogram Maximum	Yes
Autoscale Maximum Value Delay (minutes)	0.0
Autoscale Chromatogram Minimum	Yes
Autoscale Minimum Value Delay (minutes)	0.0
Fill Peaks with Color	No
Draw Grid Lines on Chromatogram	No
Show Component Fraction Numbers	No
Label with Peak Number	Yes
Label with Retention Times on Chromatogram	No
Integration Parameters	No
Starting Peak Width (seconds)	3.0
Peak Threshold	0.500
Peak Area Reject	1000
Area Reject for Reference Peaks	1000

Data Events

Time	Description
8.00	Double bunching factor

Calibration Parameters

Number Of Levels for Calibration	10
Force Calibration Curve Through Origin	No
Calibration Fit Type	Linear
Replace Or Average Calibrations	Replace
External or Internal Calibration	External
Calibrate Unknowns by Area or Height	Area
Default Injection Volume	1.0
Default Dilution Factor	1.0
Response Factor for Unknown Peaks	0.0
Calibration Standard Volume	1.0
Internal Standard Volume	1.0
Sample Unit	

Component Table -- Last Modified: 18:04 on Sun, 01 Mar 1992

Component # 1 Ascorbic Acid Retention Time 1.25
Reference Comp. none Window Size 0.15 min.
Low Amt. Limit (none) High Amt. Limit 10.00
Amount = K0 + K1*Area
K0 = -8.32793E-002
K1 = 3.39800E-004

Level	Amount	Area	Height
1	1.00000E-002	107	33
2	2.00000E-002	211	67
3	5.00000E-002	591	186
4	1.00000E-001	1046	337
5	2.00000E-001	2262	703
6	5.00000E-001	5163	1697
7	1.00000E+000	10796	3452
8	2.00000E+000	21767	7168
9	5.00000E+000	108973	14983
10	1.00000E+001	215687	29387

Component # 2 Pantothenic Acid Retention Time 1.53
Reference Comp. Ascorbic Acid Window Size 0.15 min.
Amount = K0 + K1*Area
K0 = -4.19106E-003
K1 = 2.75862E-004

Level	Amount	Area	Height
1	1.00000E-002	116	38
2	2.00000E-002	229	73
3	5.00000E-002	519	176
4	1.00000E-001	1167	368
5	2.00000E-001	2267	721
6	5.00000E-001	5679	1809
7	1.00000E+000	11243	3508
8	2.00000E+000	23498	7319
9	5.00000E+000	57705	18491
10	1.00000E+001	113468	36089

Component # 3 Riboflavin Retention Time 1.71
Reference Comp. Ascorbic Acid Window Size 0.15 min.
Amount = K0 + K1*Area
K0 = -2.53130E-002
K1 = 2.86389E-004

Level	Amount	Area	Height
1	1.00000E-002	111	33
2	2.00000E-002	221	69
3	5.00000E-002	567	185
4	1.00000E-001	1118	339
5	2.00000E-001	2266	726
6	5.00000E-001	5679	1806
7	1.00000E+000	11224	3574
8	2.00000E+000	23680	7359
9	5.00000E+000	56878	18065
10	1.00000E+001	112468	34687

Component # 4 Biotin Retention Time 2.00
Reference Comp. Ascorbic Acid Window Size 0.15 min.
Amount = K0 + K1*Area
K0 = -7.57097E-003
K1 = 2.64353E-004

Level	Amount	Area	Height
1	1.00000E-002	122	39
2	2.00000E-002	229	74
3	5.00000E-002	596	188
4	1.00000E-001	1124	372
5	2.00000E-001	2297	728
6	5.00000E-001	5836	1806
7	1.00000E+000	11886	3906
8	2.00000E+000	22806	7548
9	5.00000E+000	59687	19437
10	1.00000E+001	116899	37608

Component # 5 Niacin Retention Time 4.25
Reference Comp. none Window Size 0.15 min.
Amount = K0 + K1*Area
K0 = 3.52030E-002
K1 = 2.92375E-004

Level	Amount	Area	Height
1	2.00000E-002	211	66
2	4.00000E-002	435	139
3	1.00000E-001	1058	337
4	2.00000E-001	2035	625
5	4.00000E-001	4296	1376
6	1.00000E+000	10689	3399
7	2.00000E+000	23168	6794
8	4.00000E+000	42657	13598
9	1.00000E+001	105689	32689
10	2.00000E+001	213598	68922

Component # 6 Niacinamide Retention Time 4.60
Reference Comp. none Window Size 0.15 min.
Amount = K0 + K1*Area
K0 = 2.82227E-002
K1 = 2.80092E-004

Level	Amount	Area	Height
1	2.00000E-002	213	66
2	4.00000E-002	433	132
3	1.00000E-001	1088	345
4	2.00000E-001	2199	698
5	4.00000E-001	4359	1386
6	1.00000E+000	10989	3562
7	2.00000E+000	21898	7053
8	4.00000E+000	44689	14007
9	1.00000E+001	108936	34871
10	2.00000E+001	219874	71685

Component # 7 PABA Retention Time 5.30
Reference Comp. Niacinamide Window Size 0.20 min.
Amount = K0 + K1*Area
K0 = -1.26627E-002
K1 = 1.03601E-004

Level	Amount	Area	Height
1	2.00000E-002	191	6083
2	4.00000E-002	394	128
3	1.00000E-001	977	319
4	2.00000E-001	1948	623
5	4.00000E-001	3678	1197
6	1.00000E+000	9768	3114
7	2.00000E+000	19687	6243
8	4.00000E+000	38973	12487
9	1.00000E+001	97316	31053
10	2.00000E+001	192765	61794

Component # 8 Adenine Retention Time 5.85
Reference Comp. Niacinamide Window Size 0.20 min.
Amount = K0 + K1*Area
K0 = 1.43165E-002
K1 = 8.93715E-005

Level	Amount	Area	Height
1	2.00000E-002	216	68
2	4.00000E-002	451	149
3	1.00000E-001	1104	349
4	2.00000E-001	2211	702
5	4.00000E-001	4468	1426
6	1.00000E+000	11086	3537
7	2.00000E+000	22248	7105
8	4.00000E+000	44898	14245
9	1.00000E+001	109654	34688
10	2.00000E+001	224568	70892

Component # 9 Folic Acid Retention Time 8.72
Reference Comp. none Window Size 5.00 %
Amount = K0 + K1*Area
K0 = -1.06097E-001
K1 = 1.05674E-004

Level	Amount	Area	Height
1	1.00000E-001	986	38
2	2.00000E-001	1971	64
3	5.00000E-001	4795	153
4	1.00000E+000	9687	308
5	2.00000E+000	19053	603
6	5.00000E+000	48938	1546
7	1.00000E+001	96887	3106
8	2.00000E+001	197685	6168
9	5.00000E+001	467982	14893
10	1.00000E+002	948687	29867

Component # 10 Pyridoxamine Retention Time 12.75
Reference Comp. Folic Acid Window Size 3.00 %
Amount = K0 + K1*Area
K0 = 8.01179E-002
K1 = 1.12369E-004

Level	Amount	Area	Height
1	1.00000E-001	868	27
2	2.00000E-001	1754	55
3	5.00000E-001	4689	153
4	1.00000E+000	9067	299
5	2.00000E+000	17256	548
6	5.00000E+000	42898	1363
7	1.00000E+001	86879	2968
8	2.00000E+001	172568	5246
9	5.00000E+001	449378	14678
10	1.00000E+002	887689	26897

Component # 11 Thiamine — Retention Time 14.60
Reference Comp. Folic Acid — Window Size 3.00 %
Amount = K0 + K1*Area
K0 = -4.58504E-002
K1 = 1.03421E-004

Level	Amount	Area	Height
1	1.00000E-001	986	36
2	2.00000E-001	1904	59
3	5.00000E-001	4768	146
4	1.00000E+000	9689	311
5	2.00000E+000	19868	635
6	5.00000E+000	49687	1554
7	1.00000E+001	97818	3086
8	2.00000E+001	196848	6283
9	5.00000E+001	479683	15046
10	1.00000E+002	968738	31156

Group 1 - B Vitamins

Comp.#	Comp. Name	Ret. Time
3	Riboflavin	1.71
4	Biotin	2.00
5	Niacin	4.25
6	Niacinamide	4.60
10	Pyridoxamine	12.75
11	Thiamine	14.60

Group 2 - Other Vitamins

Comp.#	Comp. Name	Ret. Time
1	Ascorbic Acid	1.25
2	Pantothenic Acid	1.53
9	Folic Acid	8.72

Group 3 - Non-Vitamins

Comp.#	Comp. Name	Ret. Time
7	PABA	5.30
8	Adenine	5.85

Time Range(s) for Unknown Peaks

4.00 - 10.00 min.

-- DETECTOR 2 PARAMETERS --

Report Options	
Create ASCII Report File	No
Print Report	Yes
Print All Components	Yes
Print Components Found	No
Print Missing Components	No
Print All Peaks	No
Print Unknown Peaks	Yes
Print Chromatogram	Yes
Autoscale Chromatogram Maximum	Yes
Autoscale Maximum Value Delay (minutes)	2.0
Autoscale Chromatogram Minimum	Yes
Autoscale Minimum Value Delay (minutes)	2.0
Fill Peaks with Color	Yes
Draw Grid Lines on Chromatogram	Yes
Show Component Fraction Numbers	No
Label with Peak Number	No
Label with Retention Times on Chromatogram	No
Integration Parameters	
Starting Peak Width (seconds)	5.0
Peak Threshold	0.500
Peak Area Reject	1000
Area Reject for Reference Peaks	1000

Calibration Parameters	
Number Of Levels for Calibration	10
Force Calibration Curve Through Origin	No
Calibration Fit Type	Linear
Replace Or Average Calibrations	Replace
External or Internal Calibration	External
Calibrate Unknowns by Area or Height	Area
Default Injection Volume	1.0
Default Dilution Factor	1.0
Response Factor for Unknown Peaks	0.0
Calibration Standard Volume	1.0
Internal Standard Volume	1.0
Sample Unit	

Component Table -- Last Modified: 18:28 on Sun, 01 Mar 1992

Component # 1 Sodium Retention Time 2.80
Reference Comp. none Window Size 0.20 min.
Amount = K0 + K1*Area
K0 = -1.82822E-003
K1 = 1.02626E-004

Level	Amount	Area	Height
1	1.00000E-002	97	38
2	2.00000E-002	198	67
3	5.00000E-002	466	152
4	1.00000E-001	978	335
5	2.00000E-001	1947	607
6	5.00000E-001	4729	1507
7	1.00000E+000	9187	3001
8	2.00000E+000	19786	6287
9	5.00000E+000	49884	16048
10	1.00000E+001	96875	30688

Component # 2 Potassium Retention Time 3.50
Reference Comp. Sodium Window Size 0.30 min.
Amount = K0 + K1*Area
K0 = 6.28749E-003
K1 = 1.02302E-004

Level	Amount	Area	Height
1	1.00000E-002	97	31
2	2.00000E-002	197	65
3	5.00000E-002	438	143
4	1.00000E-001	973	318
5	2.00000E-001	1976	644
6	5.00000E-001	5135	1678
7	1.00000E+000	9868	3007
8	2.00000E+000	18698	6038
9	5.00000E+000	48687	14683
10	1.00000E+001	97868	29687

Component # 3 Magnesium Retention Time 7.60
Reference Comp. none Window Size 4.00 %
Amount = K0 + K1*Area
K0 = 3.67673E-001
K1 = 1.04693E-003

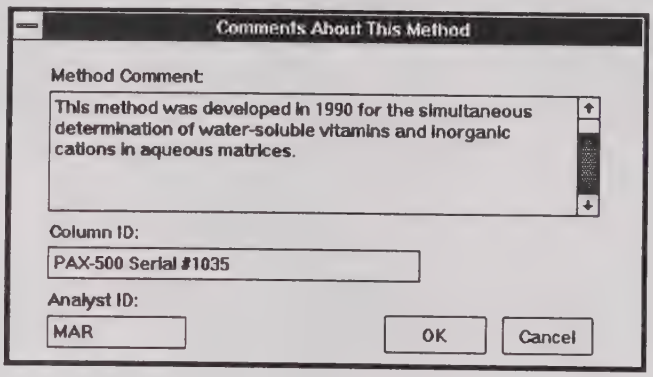
Level	Amount	Area	Height
1	1.00000E-001	91	30
2	2.00000E-001	176	57
3	5.00000E-001	453	143
4	1.00000E+000	936	305
5	2.00000E+000	1968	597
6	5.00000E+000	4807	1538
7	1.00000E+001	9076	2073
8	2.00000E+001	18739	6083
9	5.00000E+001	43798	13589
10	1.00000E+002	96781	30568

Component # 4 Calcium Retention Time 9.92
Reference Comp. Magnesium Window Size 4.00 %
Amount = K0 + K1*Area
K0 = -6.68881E-002
K1 = 1.02473E-003

Level	Amount	Area	Height
1	1.00000E-001	101	33
2	2.00000E-001	790	59
3	5.00000E-001	481	151
4	1.00000E+000	963	305
5	2.00000E+000	1902	607
6	5.00000E+000	4837	1527
7	1.00000E+001	9387	3009
8	2.00000E+001	20057	6389
9	5.00000E+001	48697	15768
10	1.00000E+002	97682	31682

Comment...

Enter any pertinent comments about the Method, as well as a description of the column and the name of the analyst, in the spaces provided in this dialog box:

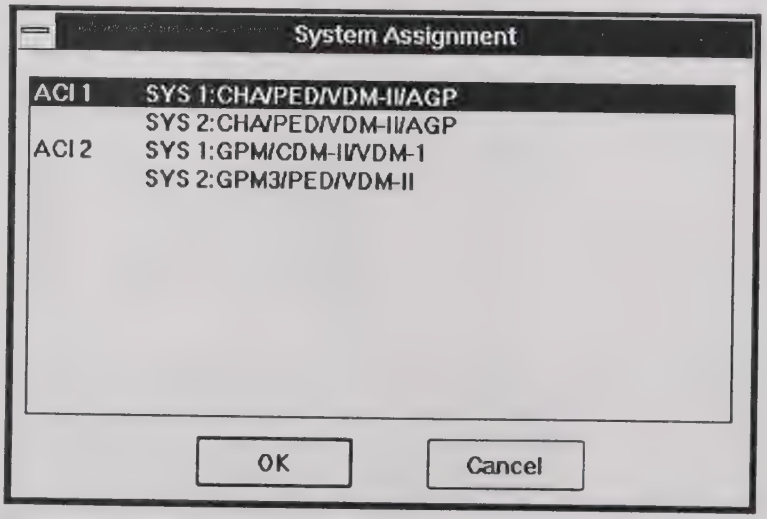


Data Acquisition Parameters

Data Acquisition parameters include all of the controls related to the operation of the analytical system and to data collection.

System

The System Assignment box shows the interface and system currently associated with the Method, along with the system description stored in the configuration file (see *Configuration Editor/AI-450 Configuration Dialog Box/System Names*). The list box shows all of the interfaces for which configurations are currently stored on disk. Tying the Method to a specific system configuration prevents the Method from being used with a configuration to which it does not apply.



When creating a new Method file, none of the other controls in the Method Editor main window are active until the system is specified. The Save and Print commands under the File menu are also inactive.

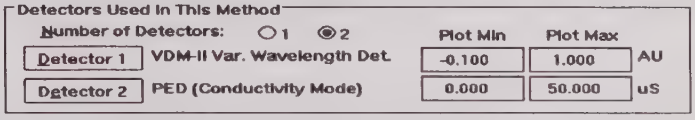
To specify the system for a Method, click on the desired interface/system in the list box and then on the OK button. The selected interface and system, along with the system description, will appear to the right of the System command button.

If you have not yet configured the interface, an error message will be displayed when you click on System. You must configure the interface, using the Configuration Editor program, before you can continue.

The System command button may also be used to change the system specification for an existing Method, as long as the new system has the same configuration as the one originally specified for the Method. If it does not, differences in the configuration may cause errors in the Instrument Control portion of the Method.

Detectors

Detector Selection



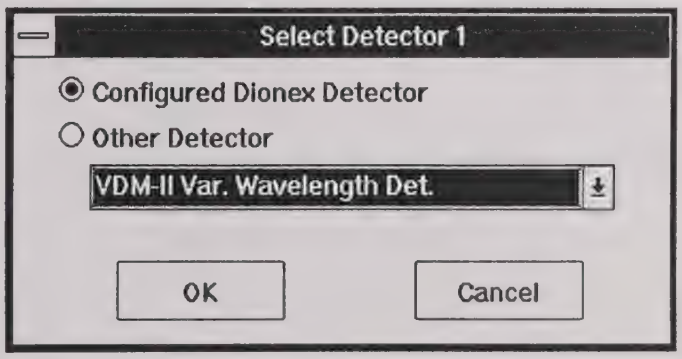
Number of Detectors specifies whether the analysis uses 1 or 2 detectors.

Plot Min and **Plot Max** are the minimum and maximum for the Y-axis of the real-time plot of the data as it is collected. The units depend on the detector selection. An inappropriate plot setting can make peaks too small to see, or else so large that the top of the peak goes off scale on the display. This setting also affects the printed output when the Autoscale options are not selected for the report (see *Method Editor/Data Processing Parameters/Report*).

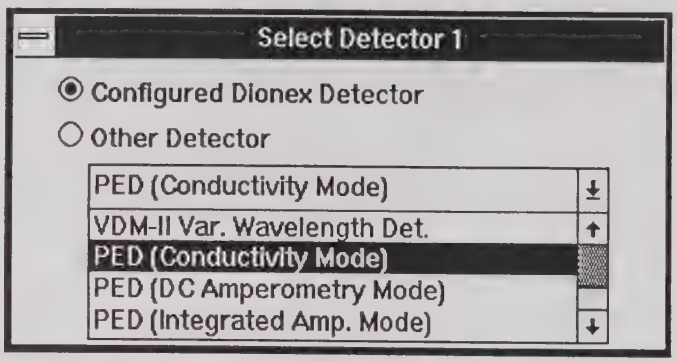
NOTE: The Plot Scale setting affects only the display of the data, not the collection of raw data from the detector.

Detector Assignment

Clicking on the Detector 1 or Detector 2 command button opens a Select Detector dialog box displaying the name of the currently selected detector. If this is the correct detector, click the mouse cursor on the OK button.



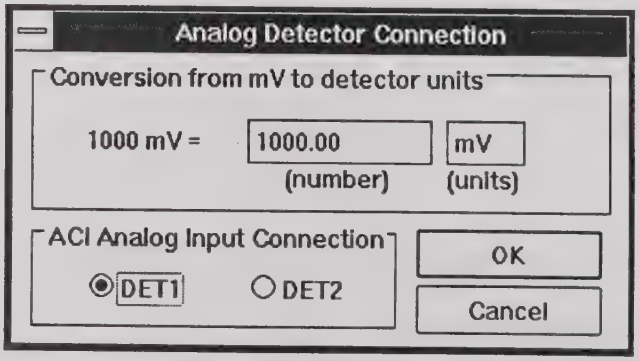
To select a different detector, click on either Configured Dionex Detector or Other Detector. Then click on the arrow at the right side of the drop-down combo box to display a list of detector names.



If you select Configured Dionex Detector, this list will comprise detectors specified in the Configuration file for the system. If you select Other Detector, several non-Dionex analog output detectors will be listed.

When you click on a detector name, the name immediately appears in the selected detector area in the middle of the dialog box. Click on OK to accept the selection.

If you select an analog detector (i.e., any detector except a Dionex Conductivity Detector Module, Variable Wavelength Detector Module-II, Pulsed Electrochemical Detector, or Capillary Electrophoresis System), the Analog Detector Connection dialog box appears:



This dialog box lets you enter a formula that the AI-450 uses to convert detector voltage output directly to detector units. For example, suppose you are using an absorbance detector set to 1 AU (absorbance unit) full scale, where full scale is 1000 mV. Type 1 in the "(number)" edit box and AU in the "(units)" edit box. When you click on OK, the AI-450 will convert the detector output directly to AU.

ACI Analog Input Connection If you use an analog detector, you must connect the Recorder output terminal on the rear of the detector to a DET connector on the ACI rear panel. Indicate which ACI connector you used by clicking on either the DET1 or DET2 button.

Select OK to accept the information you entered.

Run Time

Enter the total period of time (0.0 to 999.9 minutes) during which data will be collected, starting with the Start Sampling command from the timed events file (see *Method Editor/Instrument Control/Timed Events*). This is typically the time required for all peaks in the sample to elute. The Run Time parameter also determines the range of the Time scroll bars in the Gradient and Timed Events editors.

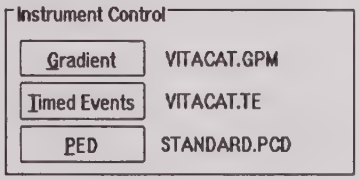
Sampling Rate

The sampling rate determines the number of data points per second (i.e., samples/second) collected and stored in the interface memory. The sampling rate can be set to 1, 2, 5, 10, 20, or 50 Hz. A sampling rate of 5 samples/second is adequate for most applications. If you have a dual-detector system, both detectors must operate at the same sampling rate.

In general, the sampling rate should be set so that each peak is defined by 20-30 data points. If you expect all of the peaks to be relatively wide at the baseline, use a slow sampling rate; if the peaks are less than a few seconds wide, use a fast sampling rate. Using a faster sampling rate than necessary does not improve accuracy; it just slows down data processing and wastes memory and disk space.

See Appendix B for more detailed information about the effect of the sampling rate on the amount of data that can be stored in ACI memory. If you have an ACI shipped before June 1, 1992, you may wish to order a RAM upgrade kit (P/N 043592) to expand the ACI memory from 64K to 256K per system.

Instrument Control



Gradient

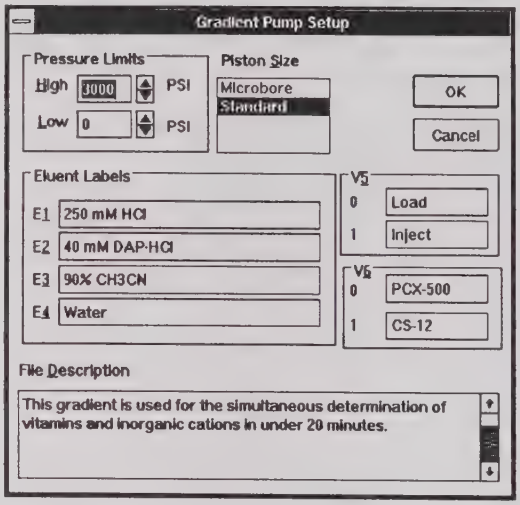
Two dialog boxes, Gradient Pump Setup and Gradient Editor, together allow you to create, save, and edit a gradient program for use by a Dionex gradient pump when the Method is run.

If a gradient file is associated with the Method, the filename and path will appear to the right of the Gradient command button. When you click on the Gradient command button to open the Gradient Editor dialog box, any existing gradient file will be automatically loaded. If no gradient file is associated with the Method, the Gradient Pump Setup dialog box will appear.

NOTE: The Gradient button is active only if the system you selected includes a Dionex gradient pump in its configuration.

Gradient Pump Setup Dialog Box

The Gradient Pump Setup dialog box can also be accessed by selecting the Pump Setup command from the Edit menu in the Gradient Editor dialog box. This dialog box allows you to set the constants that are used throughout a gradient.



Pressure Limits Use the up-down double-arrow buttons to select the high and low pressure limits, or else type the desired values in the edit boxes.

Piston Size is available only if you use an Advanced Gradient Pump (AGP). Click on the piston size, Microbore or Standard, that corresponds to your pump model.

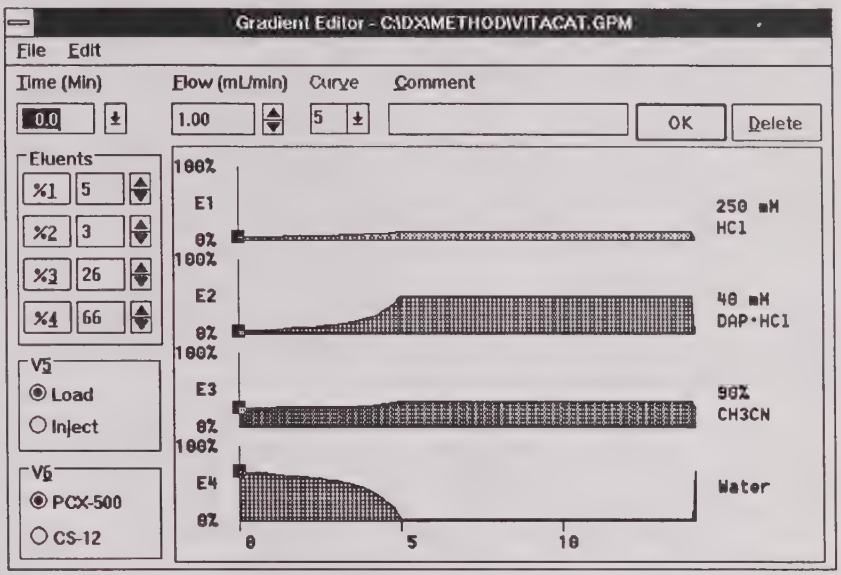
Eluent Labels Type in your own definitions for eluent labels.

File Description Type in your own description for the file.

V5 and V6 Type in appropriate labels for these two valves. Valves 5 and 6 are on/off valves typically used to control an air-operated injection valve and column select valve.

When you finish editing, select OK to save your selections.

Gradient Editor Dialog Box



File Menu

New prepares the system for creation of a new gradient file and displays the Gradient Pump Setup dialog box.

Open Gradient... displays a file Open box which allows you to select and retrieve an existing gradient file from disk.

Save Gradient... saves on disk the gradient file currently being edited. When you select this command, a Save dialog box appears. If the path shown in the dialog box is the one you want, type a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces). If you want a different path, type in the new path and then the filename.

Print sends gradient program information to your printer.

NOTE: To include a different gradient file in the Method, use the File menu command Open (to retrieve the desired file from disk) or New (to create a new file). The name of the last gradient file edited using either of these commands is included in the Method when it is saved, so be sure the correct gradient file is associated with a Method before saving it.

When using a concentration gradient, be careful to inject your sample at exactly the same point in the gradient each time. This is easily accomplished by including several minutes of your highest-concentration eluent at the beginning of the gradient program and following this with a re-equilibration step. Inject the sample at some set time after the equilibration is complete. For a complete explanation of gradient programs, refer to either the *Gradient Pump Module Operator's Manual* (Doc. 032646) or *Advanced Gradient Pump Operator's Manual* (Doc. 034463)

Edit Menu

Pump Setup displays the Gradient Pump Setup dialog box (see previous page).

Delete Step deletes the currently selected step. This command has the same function as the Delete command button in the dialog box.

Time (Min) allows you to edit or delete existing gradient steps and add new steps. A gradient file for an Advanced Gradient Pump (AGP) can contain a maximum of 32 steps; a Gradient Pump Module (GPM) gradient file can contain a maximum of 20 steps.

First, click on the down-arrow next to the Time edit box to display the gradient step times. **To select an existing step for editing,** click on that step in the drop-down edit box. **To enter a new step,** enter a new time in the edit box. **To copy an existing step,** select the step, enter a new time, and press <Enter>.

When you finish, press <Tab> to move the cursor to the Eluents section.

NOTE: Each gradient file must include initial conditions at time = 0.0. When the Advanced Computer Interface is programmed with the Method by the Run program, the gradient pump will begin operating according to the time zero step in the gradient file (see *Run/Considerations When Using a Gradient Pump*).

Eluents

Each eluent has the following controls:

A **Totalizer button** marked with the legend %1, %2, %3, or %4.

An **edit box** which displays the eluent percentage for the step being edited.

An **up-down arrow** to adjust the eluent percentage.

Before a step can be entered, the percentages of the eluents must total 100%. This can easily be achieved by using the Totalizer buttons. When you click the mouse on a Totalizer button, the percentage of the corresponding eluent will be changed to bring the sum eluent total to 100%, if possible.

Press <Tab> to move the cursor to the V5 section.

V5 and V6 Valve

Turns V5 and V6 on or off. Regardless of what function labels you give these valves, the upper position here in the dialog box corresponds to the left light on the pump front panel and the lower position to the right light.

Flow Rate

Displays the flow rate for the currently displayed gradient step. The Advanced Gradient Pump (AGP) flow rate precision is shown in hundredths of a mL/min. The Gradient Pump Module (GPM) flow rate precision is shown in tenths of a mL/min.

Use the up-down arrow on the right side of the display to change the flow rate.

Curve

This edit box, which is present only if you have an Advanced Gradient Pump (AGP), controls the gradient curve and offers the same configurations that are available from the AGP front panel. For an increasing eluent, curves 1 through 4 are convex, curve 5 is linear, and curves 6 through 9 are concave.

Click on the up-down arrow to the right of the curve edit box to open the drop-down list box and click the mouse on the desired curve number. Remember that a curve is selected at the **end** of a step, not the beginning.

Comment

Type a comment of up to 28 characters, including spaces and punctuation, for each gradient step.

OK enters the parameters selected in the Gradient Editor dialog box as a step in the gradient program being edited. Clicking on OK does not save the step as part of the file.

Delete deletes the currently selected gradient step from the gradient program.

Gradient Graph

The gradient graph updates dynamically as you change eluent proportions.

To select a gradient step, click the mouse cursor on the perpendicular step line on the graph. A darkened square, called a handle, will appear at the top of the selected step line.

To change an eluent percentage, place the cursor on the step line handle; note that the cursor arrow is now an up-down arrow. Press the mouse left button and move the step line handle up or down to change the eluent percentage. Click on the Eluents totalizer buttons as required to bring the percentage for eluents to 100%, or double-click on the makeup eluent graph.

To change the time of a gradient step, first click the cursor on the step line to select it. Next, position the cursor anywhere on the step line except on the handle (the cursor arrow will change to a right-left arrow). Now you can change the step time by depressing the mouse left button and dragging the step line left or right along the time axis.

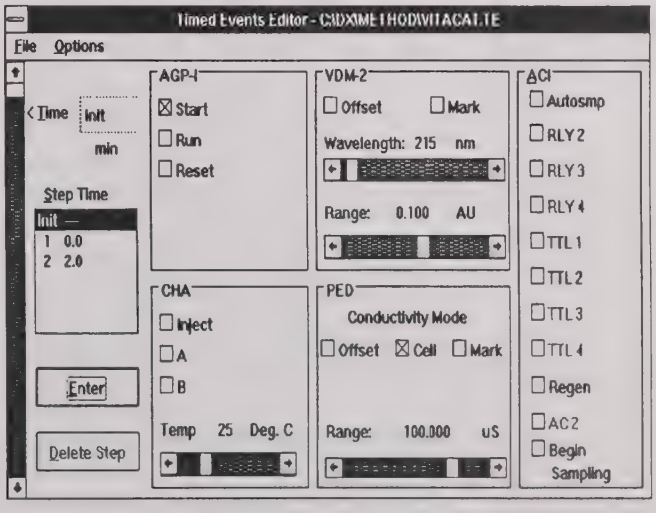
To duplicate a step at a different time, follow the procedure for changing the time of a gradient step, except now depress the <Shift> and <Alternate> keys simultaneously while dragging the time line. The position to which you drag the time line becomes a duplicate step, and the original step remains.

Timed Events

Clicking on the Timed Events command button in the Instrument Control section opens the Timed Events Editor dialog box. This editor allows you to create, save, and edit a file of timed events to control your chromatograph and its accessories.

If a timed events file is already associated with the Method, the filename will appear next to the Timed Events command button and the file, if present, will be pre-loaded automatically when you click on the Timed Events command button. If you want to include a different timed events file, either retrieve the desired file from disk (using the Open command) or create a new file (using the New command); both commands are listed on the File menu.

The **name** of the last timed events file edited using either the New or Open command is the one included in the Method. When you edit a Method, be sure the correct timed events file is associated with it.



The Dionex modules shown in the Timed Events Editor dialog box match the System configuration associated with the Method. The relays, TTL control lines, and AC outlets you assigned to the specified system will also be displayed. However, if your system is configured with a relay-only Data System Model 1R or 2R, the controls portion of the Timed Events Editor dialog box will be blank except for the fields noted below. RLY x, TTL x, and, for Model 2R only, AC x, are default names. If you entered different names in the Configuration Editor, those names will be shown instead.

Because the number of modules the AI-450 software can control is continually increasing, controls for individual modules are not described in this manual. Instead, refer to the operator's manual for specific modules for detailed descriptions of all controls.

Enter adds the conditions selected for each module at a specified time as a step in the Timed Events table. **If the time for a new step is the same as that of an event already in the table, the new parameters will replace the old parameters.**

Time The scroll bar on the left side of the editor sets the time, in tenths of minutes, at which selected conditions will go into effect. Each time entry is a separate step in the Timed Events table. The range of the scroll bar depends upon the Run Time specified.

Step Time displays the step number and time of each entry in the Timed Events table. To select a particular step in the table, locate the step in the list box and click on it. The conditions currently stored for that step will be displayed on the control panel.

Every timed events file must include an initial (INIT) conditions step. The INIT step typically contains standby conditions for each module in the system. This allows you to include system equilibration conditions in each Method. The conditions in the INIT step are in effect from the time the Method is downloaded to the interface until the time the Method is started.

The conditions in the time 0.0 step go into effect as soon as the interface receives a START command. The conditions in the time 0.0 step need not match those in the INIT step.

When the next analysis starts, the time 0.0 step executes immediately after START is selected.

Delete Step removes the selected step from the Timed Events table. Select the desired step from the Step Time list box, then click on Delete. The remaining steps will be renumbered consecutively. The INIT and time 0.0 steps cannot be deleted.

ACI Controls Refer to the *Advanced Computer Interface Manual* (Doc. 034011) for details.

RLY x, where x is a number from 1-4, controls the relays assigned to the system for which the Method is written. These relays provide contact-closure control of accessories such as a stream-select valve.

TTL x, where x is a number from 1-4, controls the TTL outputs assigned to the system for which the Method is written. These outputs provide control of accessories that need only a digital signal to trigger their operation.

AC x, where x is a number from 1-2, controls the AC outlets assigned to the system for which the Method is written. These outlets provide programmable AC power connections for accessories that require simple on/off control (e.g., a recorder or a sampling pump). Each outlet is rated for 2 amps.

Begin Sampling starts collection of detector data by the ACI. To issue a new Begin Sampling command, you must first end the current chromatogram.

File Menu

Save...

Saves on disk the timed events file currently being edited. When you select this command, a Save dialog box will appear (see *Method Editor/File Menu/Save and Save As...*).

Options Menu

List

Displays all the operating conditions listed in the Timed Events table, as well as the times associated with them.

EXAMPLE:

Enter a Timed Events table for a system consisting of a GPM (Gradient Pump Module), CHA (Advanced High- Pressure Chromatography Module), Conductivity Detector Module (CDM), and an ASM (Automated Sampler).

Time = Init
Flow rate = 0.5 mL/min
E1 = On
E2-6 = Off
Valve B = On
Inject valve = Off (Load)
Cell = On
Range = 10 µS

Time = 0.0
Relay 1 = On (trigger autosampler LOAD sequence)

Time = 1.0
CDM Offset = On

Time = 1.1
Inject valve = On (Inject)
Mark = On

Time = 1.2
Relay 1 = Off

1. Click on the Timed Events command button to open the Timed Events Editor dialog box.
2. Verify that the INIT step is selected.
3. Point and click on the E1 check box in the GPM section. Point and click on the left arrow of the Flow scroll bar until the flow rate is set to 0.5 mL/min.
4. Click on Enter to enter the initial conditions step in the timed events table.
5. Using the Time scroll bar, set the time to 0.0.
6. Repeat Step 3 to set the Time 0.0 conditions, then click on the Relay 1 check box in the ACI section. Click on Enter to enter the time 0.0 conditions as Step 1 in the timed events table.
7. Using the Time scroll bar, set the time to 1.0. Click on the Offset check box in the CDM section and then click on Enter.
8. Continue the process described in Step 7 to enter the remaining timed events.

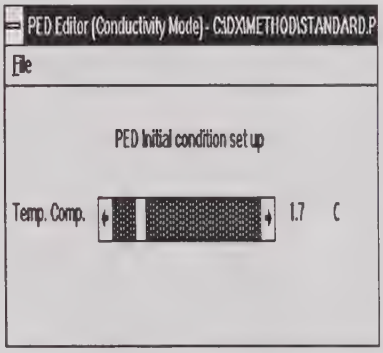
9. Verify that the conditions for each step are correct by selecting each step from the list box and examining the conditions as they appear on the control panel.

10. Select Save (from the File menu) to save the timed events file. Type the filename AS5A in the filename box and click on Save. The extension ".TE" is added to the filename automatically and the file is added to the Method being edited. Add the file to the directory of your choice.

PED Editor

Click the mouse cursor on the PED command button to open the PED Editor. One of the next three dialog boxes will appear, depending upon which PED Mode you selected in the Detectors Assignment section.

Conductivity Mode



File Menu

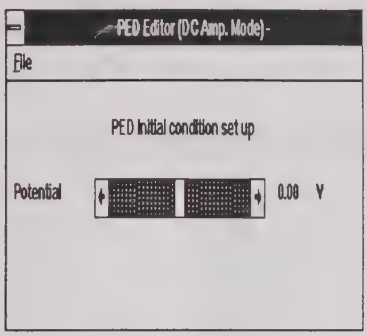
New prepares the system for creation of a new file.

Open... presents a file box from which you can select and retrieve an existing file from disk.

Save... saves on disk the PED file currently being edited and opens a Save dialog box in which you type a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces).

The dialog box scroll bar adjusts the detector temperature compensation factor to correct for changes in response caused by temperature variation in the mobile phase. For more information about temperature optimization, see the *Pulsed Electrochemical Detector Operator's Manual* (Doc. 034177).

DC Amperometry Mode



File Menu

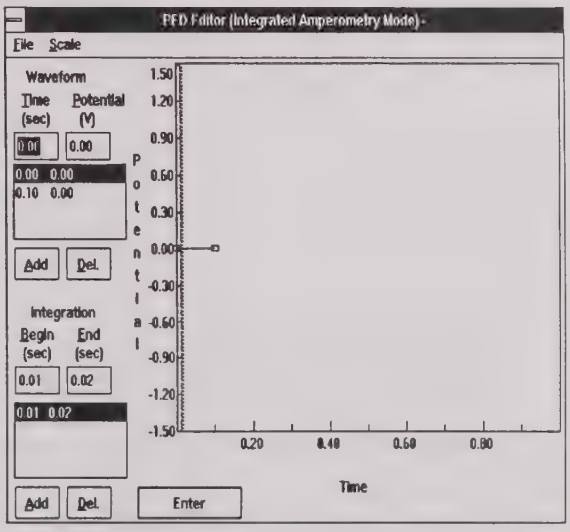
New prepares the system for creation of a new file.

Open... presents a file box which allows you to select and retrieve an existing file from disk.

Save... saves on disk the PED file currently being edited. When you select this command, a Save dialog box appears. Type in a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces).

The DC Amperometry Mode can be used for either DC amperometry or cyclic voltammetry. The dialog box scroll bar allows you to select a potential for a new program or edit a potential in an existing program. For detailed information about how to program the PED, see the *Pulsed Electrochemical Detector Operator's Manual* (Doc. 034177).

Integrated Amperometry Mode



File Menu

New prepares the system for creation of a new file.

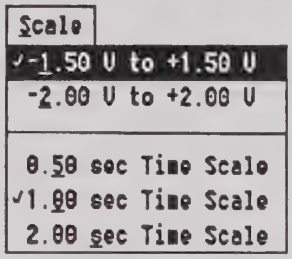
Open... presents a file box in which you can select and retrieve an existing file from disk.

Save... saves on disk the PED file currently being edited and opens a Save dialog box. Type in a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces).

Print sends the current file to the printer.

Scale Menu

Select the graph scales you want to view by clicking the mouse cursor on your choices.



Creating a Program

The Integrated Amperometry Mode can be used for integrated amperometry or pulsed amperometry. To create a program, you must first enter both a time/potential program and an integration period. The repeating sequence of potentials applied to the cell working electrode is defined by points selected on the displayed graph.

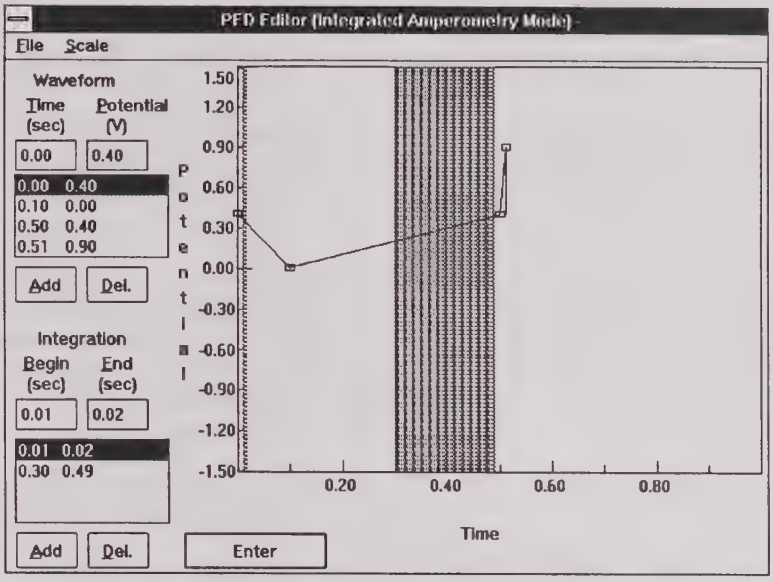
Step 1 of every program is at time zero. Since this cannot be changed, the first value you will enter is the first potential. Enter the remaining times and potentials in sequence until the program is completed; for each step, enter first the time and then the potential. Enter a program as follows:

Waveform

1. Enter the potential for the first step of the program in the Potential dialog box (the Waveform Time is always 0 for the first entry). Click on the Add command button below the Waveform list box to enter the value.
2. Enter the time for the second step in the Time edit box. Use the <Tab> key to move to and highlight the Potential edit box, then enter your second step Potential value. Click the mouse cursor on the Add button to enter the values. (If you prefer, use the I-bar cursor to enter values in the edit boxes rather than highlighting with the <Tab> key.)
3. Enter the remaining times and potentials in sequence until the program is complete.

Integration

1. Enter the Integration beginning time and ending time in the Integration dialog boxes. Click the mouse cursor on the Add command button.
2. Notice that the integration period appears as a shaded region on the screen waveform graph.



Editing a Program

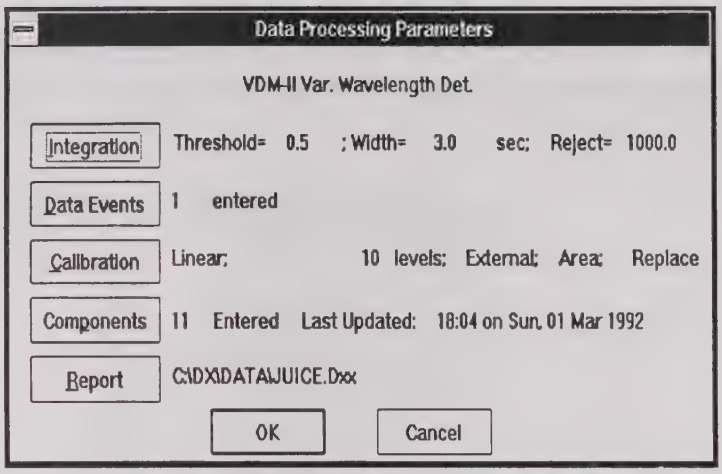
To edit a program:

1. Select a line in the list box for editing.
2. Change the value(s) as desired in the edit box(es).
3. Click the mouse cursor on the Enter command button.

Data Processing Parameters

Detector 1 or Detector 2

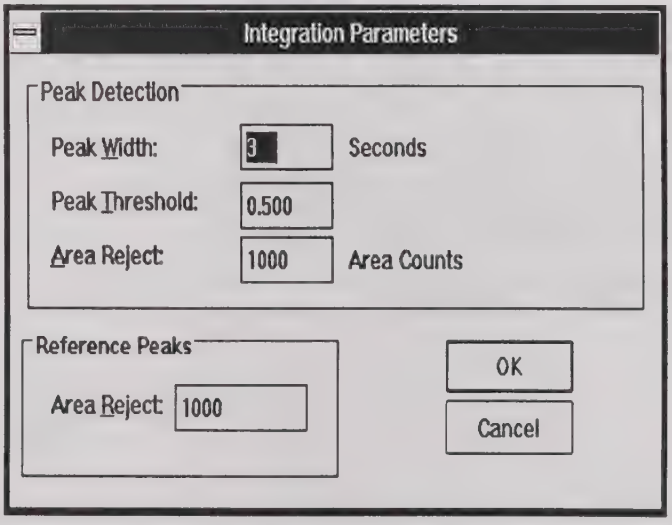
These command buttons open the Data Processing Parameters dialog box for the selected detector.



Data processing parameters specify how the data from an analysis is processed, what information is included in the final analytical report, and whether the data is stored on disk. Clicking on any of the five command buttons here will open a dialog box in which you select data processing parameters. Each of these dialog boxes is described in one of the following sections.

NOTE: The easiest way to edit Data Events is through the Optimize program, since the effects on integration are displayed instantly.

Integration



Integration parameters specify how the acquired data is processed by the computer for peak detection and integration. Refer to Appendix B for detailed information about the principles of peak integration.

Peak Width and sampling rate (samples/second), establish how raw, digitized data samples are averaged (or "bunched"). Bunching helps separate electronic noise from the true signal. The data acquisition software bunches 10 digital data points for each multiple of the peak width value.

A peak width of 5-10 seconds is satisfactory for most LC applications. If narrow peaks of interest to you are not being detected, reduce the peak width. If baseline

noise is being detected as peaks, increase the peak width and/or the peak area reject value.

If the peak widths are narrow at the beginning of an analysis and much wider at the end, or if an eluent change results in late- eluting peaks being narrower than earlier ones, you can elect to double or halve the bunching factor at a specified time in the analysis (see *Method Editor/Data Processing Parameters/Data Events*).

Peak Threshold specifies the threshold value (counts per data point interval) for peak detection.

The peak threshold is a measure of how fast the leading edge of the peak must rise before it is recognized as a peak. The larger the number (slope), the sharper the peak must be. Peaks that have slopes below this threshold are not integrated or reported, but they are stored as raw data.

Basically, the peak threshold provides filtering to prevent long-term baseline drift from being detected as peaks. If drift or baseline noise is being detected as broad peaks, increase the peak detection threshold. Use the Auto Threshold feature in Optimize to set the peak threshold (see *Optimize/Operations Menu/Auto Threshold*). See Appendix F for an example of how adjusting the peak width and peak threshold can affect analytical results.

Area Reject specifies the minimum area a peak must have before it is included in the final report.

If small peaks of interest are not being reported, decrease the Area Reject value. If you want to have all detected peaks listed on the final report, enter a value of zero.

The raw data comprising rejected peaks is stored on disk along with the data for unrejected peaks. Rejected peaks can be included in a later report by detecting the stored raw data again and changing the peak area reject value.

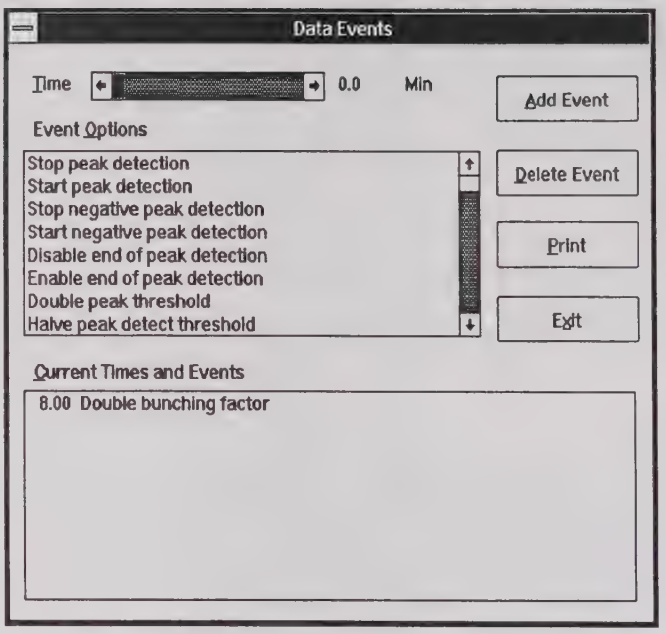
Area Reject (Reference Peaks) specifies the minimum area a peak must have before it is recognized as a reference peak.

A reference peak is typically an early-eluting peak used to help compensate for systematic variations in retention times for each injection. The variation in reference peak retention times is used to determine the expected variation in the retention times of subsequent peaks. For particularly long analyses, you may want to use two or more reference peaks.

Data Events

Data Events are time-based events which affect how raw data is detected and integrated. Many analyses require special treatment of unresolved peaks or baseline phenomena in order to ensure that the peaks are integrated correctly; the void volume peak found in many analyses is a good example. In other analyses, late-eluting peaks are significantly broader than earlier peaks. For these peaks, the bunching rate set at the beginning of the analysis must be changed to ensure accurate integration.

It is best to run a Method once **without any Data Events** to determine exactly which events are needed and when they should occur. You can use the controls in the Data Events dialog box to create a list of up to 100 events for each Method. The easiest way to edit these events is through the Optimize program, since the effects on integration are displayed instantly.



Time Use the scroll bar to set the time at which the selected Data Event will occur. The maximum selectable time is determined by the Run Time specified for the Method.

Add Event adds the item highlighted in the Event Options window to the Current Times and Events window.

Delete Event deletes the highlighted event from the Current Times and Events window.

Print prints the Integration Timed Events portion of the Method Parameters Report.

Exit closes the Data Events dialog box.

Event Options Use the scroll bar to view all of the Data Event options. To select an option, point and click. The event description will be highlighted.

Stop and **Start peak detection** toggle peak detection on and off. This is especially useful for preventing spurious reproducible changes (e.g., those caused by an eluent change or a large pressure change) from being detected as peaks.

Stop and **Start negative peak detection** turn a special negative peak detection algorithm on and off so that negative peaks can be detected.

Disable and **Enable end-of-peak detection** turn the end-of-peak detection function on and off. The end of a peak is normally identified when three consecutive data points fail to decline by the peak threshold value. This option is especially useful for controlling the determination of the end of a peak with a long tailing edge.

Double and **Halve peak detect threshold** multiply or divide the current peak threshold by two.

Doubling the threshold can be useful when an eluent change or a gradient causes the width of late-eluting peaks to be narrower than that of peaks at the beginning.

Halving the threshold is useful when late-eluting peaks are significantly broader than early-eluting peaks. See Appendix B for additional information about the peak threshold.

Double bunching factor doubles the number of points averaged to make a bunch. The initial bunching factor is determined by the Peak Width value specified in the Integration parameters. In effect, each time the bunching factor is doubled, the previously set peak width value is doubled. See Appendix B for more information about bunching.

Force baseline at start of this peak forces a baseline at the start of a peak where the peak detection software does not normally find a baseline.

Force baseline at start of all peaks forces the peak detection software to generate separate baselines, valley-to- valley, for each peak in the chromatogram.

Force common baseline following this peak forces a common baseline for all peaks following the specified peak. The peaks following this command will be separated by exponential projections or drop lines, as appropriate.

End and **Don't end peaks on baseline penetration** turns the end-of-peak-on-baseline- penetration function on and off. The end of a peak is not normally detected upon baseline penetration.

Halve bunching factor divides in half the number of raw data points that are combined to form a bunched point. The initial bunching factor is determined by the Peak Width specified in the Integration parameters. Halving the bunching factor essentially halves the previously set Peak Width value.

Void volume treatment for this peak automatically forces a horizontal baseline from either the beginning or end of a peak to the data point closest to zero on the edge of a dip or negative peak. Enter the retention time of the peak requiring this treatment as the time for the event.

Disable and **Enable exponential skim** turn on and off the function that forces the baseline of a shoulder peak not normally fitting into baseline category three or four (because it has an area greater than 25% of the major peak) to be determined by exponential projection.

Current Times and Events displays the selected Data Events.

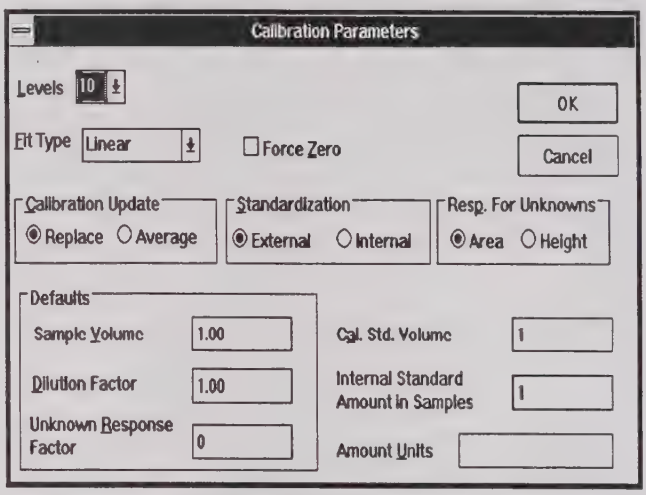
Correcting Baselines with Void Peaks

Void peaks cause the peak detection algorithms to find an inappropriate baseline (i.e., one that starts from the most negative point of the dip and ends after the void upset). If you want to quantify peaks that elute just after the end of the void, use the following procedure to correct the baseline.

1. Click on the Data Events command button in the Data Processing Parameters dialog box to open the Data Events dialog box.
2. Use the Time scroll bar at the top of the Data Events dialog box to select a time within the first peak following the dip.
3. Move the Event Options scroll bar down until you find the Void Volume Treatment option; click on the option to highlight it.
4. Click on the Add Event command button. The time and event description will appear in the Current Times and Events window.

Calibration

Calibration parameters determine how detected peaks are identified and quantitated. Refer to Appendix C for detailed information about the principles of peak quantitation.



Levels (1 to 10) specifies the total number of different concentration calibration standards to be used in calculating concentrations.

Many calibrations are single-level (i.e., use only one calibration standard). A single-level calibration assumes that the detector response for all sample components is linear over the entire amount range. If this is not the case, use a multilevel calibration.

In multilevel calibrations, several standards of different amounts are used to construct a calibration curve for each sample component. Data from each calibration run is saved until responses for each calibration level have been measured for each component. This calibration data is then used in either a linear, point-to-point, quadratic, or cubic fit to construct the calibration curve for each component. The component amounts in a sample mixture can then be determined by relating the measured areas (or heights) to their corresponding calibration curve.

Fit Type (Linear, Pt. to Pt., Quadratic, or Cubic) selects a fit type for the calibration curve. See Appendix C for a detailed discussion of curve fitting.

A **Linear** fit can be used with one or more calibration levels. If more than one level is used, the calibration curve is determined by a least-squares calculation to fit a line through the calibration data points. If only one calibration level is used, the curve is forced through the origin (zero).

A **Point-to-point** fit can also be used with one or more calibration levels. If more than one calibration level is used, the software determines which two calibration data points bracket the response of the unknown and then uses a straight line between these two calibration data points to interpolate the concentration of the unknown. If only one calibration level is used, the line is drawn from the data point on the graph through (0,0).

Quadratic and **Cubic** fits can be used only with multilevel calibrations. A quadratic fit requires at least three calibration levels, while a cubic fit requires at least four calibration levels. The calibration curve is drawn from a quadratic or cubic equation. A quadratic or cubic fit is most useful when detector response is known to "flatten out" toward one extreme of the concentration range or the other.

Force Zero determines whether the curve goes through the origin of the plot. This option is available only when the fit type is linear or point-to-point.

Calibration Update (Replace or Average)specifies the method used to update the calibration table for each component. The calibration table includes such information as the retention times, peak areas, peak heights, and response factors determined for each component during calibration. The table can be updated during subsequent calibrations in one of two ways: by replacing the existing values in the table with new values or by averaging new values with the existing values.

Standardization (External or Internal) specifies whether peaks are quantitated using internal standard or external standard calculations. See Appendix C for details.

Resp. for Unknowns (Area or Height) specifies whether quantitation of unidentified peaks will be based on the areas or heights of the peaks.

Default Sample Volume specifies the injection volume for the sample. For external standard calculations, this value is used as a divisor for component amounts. If all injections have the same volume, enter a value of 1. (This value is not used for internal standard calculations.)

Default Dilution Factor specifies a multiplier to be used during amount calculations to correct for differences in dilution and sampling procedures from one sample to the next. The factor is determined by dividing the diluent volume by the sample volume. If all samples are prepared in the same way, enter a factor of 1. (This value is not applied when calibration standards are injected.)

Default Unknown Response Factor specifies the response factor to be applied to unidentified components in a sample in order to calculate an approximate concentration. Response factors are used during an external standard calibration to correct for the differences in detector response for different compounds.

> Detector response varies from one compound to another. During calibration, correction factors are calculated which measure the relative detector response for each known sample component. These correction, or response, factors are used when calculating the response-corrected concentrations of each sample component.
>
> The response factor for an unknown peak must be determined experimentally. Its value has no effect on the quantitation of known peaks. See Appendix C for detailed information about the calculation of response factors for both internal and external standards.

Cal. Std. Volume (Calibration Standard Volume) specifies the injection volume for the standard(s). This value is a multiplication factor used to calculate external standard calculations of component amounts. (This value is not used for internal standard calculations.)

$$\textit{Multiplication Factor} = (\textit{Cal. Std. Volume} / \textit{Sample Volume}) \times \textit{Dilution Factor}$$

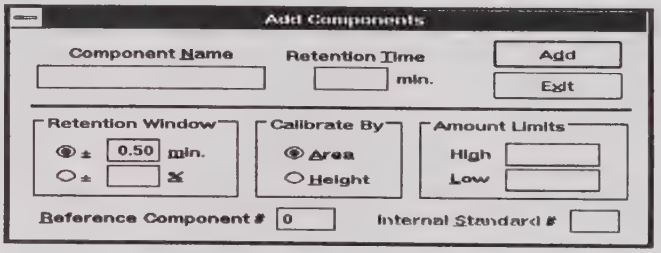
Internal Std. Amount in Samples specifies the default amount of the internal standard in the sample. (If you are using an external standard, this parameter is not used.)

Amount Units specifies the unit of measure (e.g., ppm, mg/mL, or nanomoles) used to express the quantity of sample components.

Components

If you want sample components to be identified and quantitated automatically, you must first create a Component Table. The Component Table contains the information, including the name, concentration, and retention time of each sample component, that is needed to perform calibration calculations.

When you click on the Component button in the Data Processing Parameters dialog box, the Add Components dialog box appears, superimposed over the Component Table:



After entering the component's name and data, click on Add to add the component to the Component Table. Repeat this procedure to enter all new components in the table.

Component Name Enter a component name of no more than sixteen characters. The component name will be included on the chromatogram and printed in the Result Report, if you want (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Report*). The Component Table can contain a maximum of 150 components.

Retention Time Enter the expected retention time (in minutes) for the component. If you do not know the retention time, enter any number greater than zero. The correct retention time can be determined later from the first calibration run, and the Component Table then updated. In subsequent calibrations, the retention time will be updated automatically either by averaging or replacing, depending on which method you selected in the Calibration Parameters dialog box (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Calibration*).

Retention Window (min. or %) determines the amount by which the retention time of the specified peak can vary and still be identified. If more than one peak appears within the component time window, the peak closest to the adjusted retention time is selected. It is usually advisable to keep the window as narrow as possible in order to exclude unwanted peaks. The retention window can be expressed in one of two ways:

min. specifies the window size as an absolute value, in minutes.

% specifies the window size as a percentage of the expected retention time, in which a peak must appear before it can be identified. A 3% window is usually satisfactory.

If more than one peak appears within the window for the **reference component**, the **largest peak** is selected as the reference. If the true experimental retention time of the reference peak differs from its expected time, the data acquisition software proportionally adjusts the expected retention times of the other components.

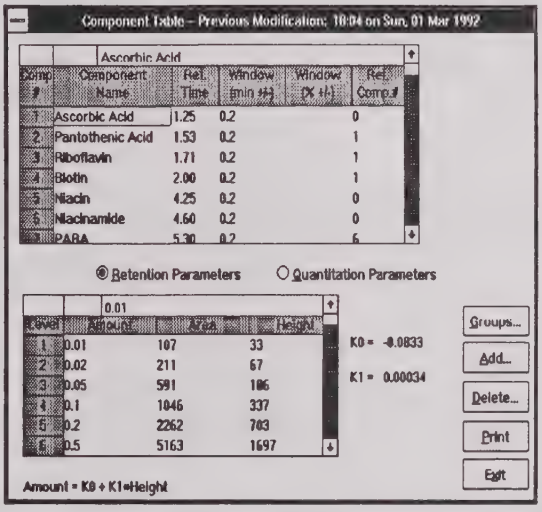
Calibrate By specifies whether each component is calibrated by peak area or peak height.

Amount Limits specify high and low limits for the calibration method. Amounts exceeding these limits are annotated on the report.

Reference Component # You may enter the number of a reference component to use to adjust the retention time of the current component. If you enter a number, the expected retention time of the current component will be adjusted **proportionally** to shifts in retention of the reference component. If you leave the default number (0), no adjustment will be made.

Internal Standard # If the internal standard was selected in the Calibration Parameters dialog box, enter the number of the component that you want used as the internal standard.

When you finish editing in the Add Components dialog box, click the mouse cursor on the Exit command button to open the Component Table dialog box:


Component Table – Previous Modification: 18:04 on Sun, 01 Mar 1992
Ascorbic Acid
Comp. # | Component Name | Rel. Time | Window (min +/-) | Window (% +/-) | Ref. Comp.#
1 Ascorbic Acid 1.25 0.2 0
2 Pantothenic Acid 1.53 0.2 1
3 Riboflavin 1.71 0.2 1
4 Biotin 2.00 0.2 1
5 Niacin 4.25 0.2 0
6 Niacinamide 4.60 0.2 0
7 PABA 5.30 0.2 6
Retention Parameters
Quantitation Parameters
0.01
Level | Amount | Area | Height
1 0.01 107 33
2 0.02 211 67
3 0.05 591 186
4 0.1 1046 337
5 0.2 2262 703
6 0.5 5163 1697
K0 = -0.0833
K1 = 0.00034
Groups...
Add...
Delete...
Print
Exit
Amount = K0 + K1*Height

Click on **Retention Parameters** to display the values selected for Retention Time, Retention Window, and Reference Component Number for each component in the table (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Calibration*).

Click on **Quantitation Parameters** to display the values selected for Retention Time, Calibrate By, Low Limit Amount, and High Limit Amount (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Calibration*).

Comp. # and **Component Name** identify which component in the Component Table is being edited.

Level indicates which calibration level is being edited.

Amount is the component amount at the corresponding calibration level. The concentration must increase as the level number increases.

Area and Height Use these sections to edit area and height values manually, if desired. This is not usually necessary because the Run and Batch programs perform automatic calibrations when standards are analyzed. In addition, Optimize has a semiautomatic calibration utility.

The following values are used to calculate the amount of a component. They are all determined automatically and cannot be edited.

K0 indicates the Y-intercept of the calibration curve.

K1 is the coefficient for the first-degree variable. When the fit type is linear, K1 indicates the slope of the calibration curve for the selected calibration level.

K2 is the coefficient for the second-degree variable in a quadratic fit.

K3 is the coefficient for the third-degree variable in a cubic fit.

For example, to determine the cubic fit of peak area calibration, the equation is:

$$Amount = K0 + K1 \times Area + K2 \times Area^2 + K3 \times Area^3$$

Print prints the Component Groups section of the Dionex Method Parameters Report.

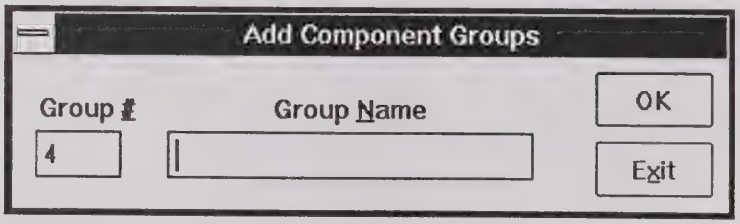
Add reopens the Add Components dialog box (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Components*).

Delete deletes the component highlighted in the list box. Components cannot be deleted if they are being used as references.

Print prints the Component Table section of the Dionex Method Parameters Report.

Exit returns you to the Data Processing Parameters dialog box.

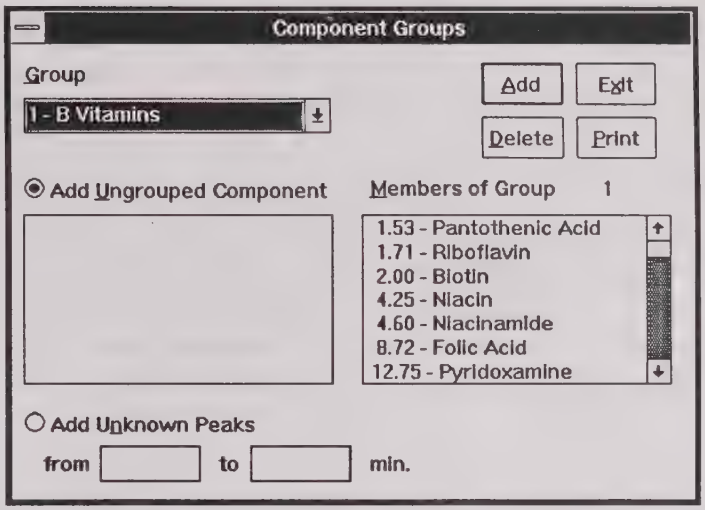
Groups... This option opens a series of dialog boxes that allows you to classify components into groups. If no groups exist, the Add Component Groups dialog box appears first:



A component group can contain any number of components and as many as five different time ranges. Enter a group number (1 to 20) and a group name in the edit boxes here and click on OK to enter the data in the Component Table. Repeat this procedure for each component group.

If you need to insert a new group between two existing groups in the Component Table, simply enter the number and name of the new group and click on OK. The groups that follow the new group will automatically be renumbered.

When you finish adding component groups, click on Exit and the Component Groups dialog box will appear:



Group displays the name and number of the component group being edited. To select a different group, click the mouse cursor on the down- arrow, then click on the desired name in the list box of component groups.

Add Ungrouped Component displays the names and retention times of all ungrouped components. To add an ungrouped component to the group being edited, click on the component name, then click on Add to transfer the highlighted component name to the Members of Group window. Note that each component can belong to only one group.

Add Unknown Peaks adds unknown peaks that appear within a particular time range to the component group being edited. Enter the beginning and end of the time range (in minutes) in the edit boxes, then click on the Add command button to add the word "unknowns" and the time range to the Members of Group window. Double-clicking on an item in this window will also transfer it to the Members of Group window.

Members of Group lists the components (preceded by their retention times) that belong to the component group being edited. Time ranges entered for unknown peaks will also appear here. The number after the label is the number of the group. Double-clicking on an item in this window will transfer it to the Add Ungrouped Component window.

Add removes the highlighted component in the Add Ungrouped Component window and transfers it to the Members of Group window. Or, if there is no highlighted component, clicking on Add opens the Add Component Groups dialog box.

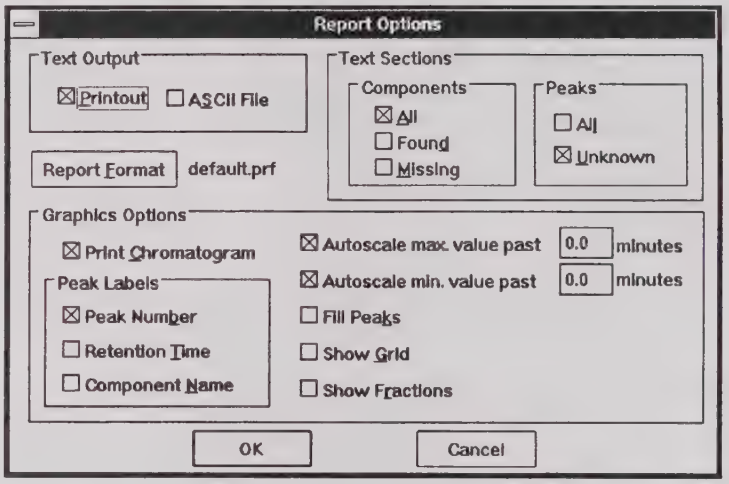
Delete If there is a highlighted item in the Members of Group window, clicking on Delete removes it from the group (but not from the Component Table. Or, if the Group name is highlighted, clicking on Delete deletes the entire group.

Print prints the Component Groups portion of the Dionex Method Parameters Report.

Exit closes the Component Groups dialog box and returns you to the Component Table.

Report

A report lists the retention times, names, areas, heights, and calculated concentrations for all peaks detected in an analysis. Use the Report Options dialog box to select a report's parameters.



Text Output

Printoutprints a report, without a chromatogram. Note: The report can be saved even if you do not print it.

ASCII File stores the text of the report on disk as an ASCII file. The file is stored under the same filename used for the raw data, but with the extension ".Rxx", where xx is a number from 01 to 99.

Report files are generally used only to transfer results to other programs (e.g., a database). ASCII files take up a substantial amount of disk space, so select this option only when necessary. You can also create an ASCII report file later, from a data file, using the Optimize or Batch program.

Report Format

The AI-450 software supplies a default format file, DEFAULT.PRF, which can be used to print a report with peak number, retention time, component name, amount, height, area, baseline type, and % delta retention time. If you do not want to use the default format, click the cursor on Report Format to display an Open box and select one of several other formats also provided.

NOTE: If you prefer to create customized report formats, a separately sold AI-450 accessory program, Form Editor (P/N 043020), is available for that purpose.

Text Sections

Components Options

Checking any of the following boxes will generate a separate table in the report.

All Components creates a list of every component in the Component Table, regardless of whether it was detected during the analysis.

Found Components creates a list of only those components in the Component Table that were detected during the analysis.

Missing Components lists the names of any components that were listed in the Component Table but not detected during the analysis.

All Peaks lists all peaks in the chromatogram, regardless of whether they were entered in the Component Table.

Unknown Peaks lists any peaks that were detected but could not be identified.

Graphics Options

Print Chromatogram prints the chromatogram, including baselines and retention times.

Autoscale max. value past sets the top of the response axis to the maximum value detected past a certain time; enter that time in the edit box here.

Autoscale min. value past sets the top of the response axis to the minimum value detected past a certain time; enter that time in the edit box here.

Fill Peaks opaques the area enclosed by the plotted peak.

Show Grid superimposes a grid over the chromatogram.

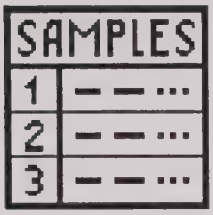
Show Fractions shows the fractions collected by the fraction collector (if one was used) on the chromatogram.

Peak Labels

Peak Number prints a numbered label on each peak in the chromatogram.

Retention Time prints the retention time for each peak in the chromatogram.

Component Name prints component names next to the peaks with which they are associated.



The Schedule Editor program is used to create, edit, and save Schedules. A Schedule consists of one or more Methods linked together to perform a series of analyses automatically and may include, for instance, one or more calibration runs, analyses of several samples, a column change and an equilibration, followed by new calibration runs and more samples. Each Schedule step specifies the Method, data filename, and other parameters to be used to analyze a single injection. The Schedule Editor program can also be used to link two or more Schedules so that they automatically run sequentially.

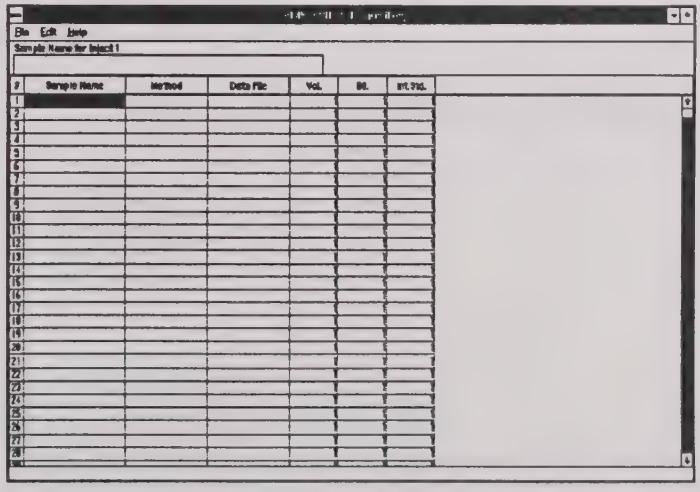
When you use a Schedule, the Run program retrieves the Schedule file from disk and uses the first Method in the Schedule to analyze the first injection. The Advanced Computer Interface (ACI) and gradient pump are programmed with the Control and Gradient sub-files stored with the Method and raw data is processed according to the data processing parameters set for the Method.

After executing the first Method and printing any reports you requested, Run loads the Method for the second injection. If this is not the Method used for the first injection, Run will transfer the new Method to the interface. Any Control timed event of a previous run not being executed will be void.

This process is repeated for each step of the Schedule. The entire Schedule may be repeated from 1 to 99 times or indefinitely.

Schedules are also used to specify groups of data files for reprocessing, using the Batch program.

Starting the Schedule Editor Program



To start the Schedule Editor program, double-click on the Schedule icon in the AI-450 MainMenu or on SCHEDULE.EXE in the directory that holds your AI-450 files. The Schedule Editor's main window will appear. The main window contains an empty Schedule in the form of a blank worksheet. Each step (row) in the worksheet represents one injection. There are three pull-down menus. The commands, dialog boxes, and windows associated with each menu are explained on the following pages.

SCHEDULE.EXE Command Summary

File Menu
- New
- Open...
- Save
- Save As...
- Print..

Edit Menu
- Insert...
- Delete...
- Copy...
- Comment...

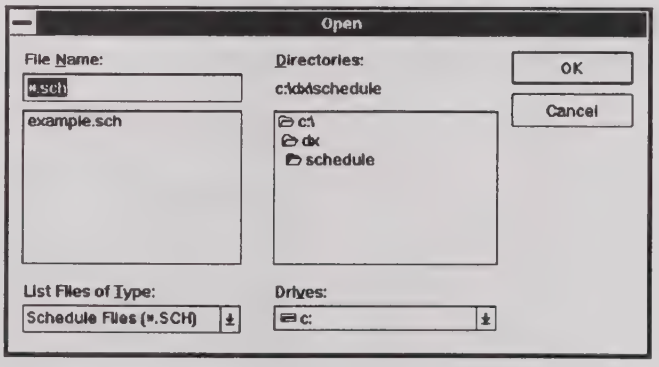
File Menu

New

The New command clears any information currently displayed on- screen and sets up the worksheet for a new Schedule.

Open...

Use this command to open an existing Schedule and display it in the main window.

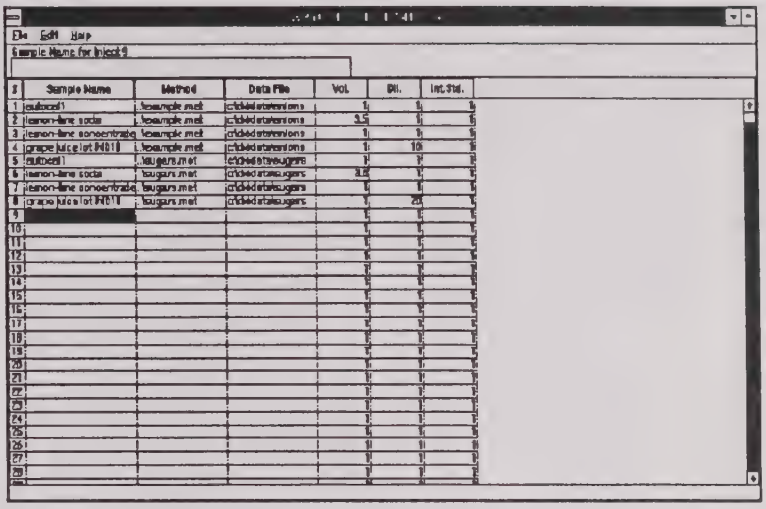


Schedule Editor Worksheet

A Schedule can contain instructions for up to 99 separate injections. The last Schedule step that includes a Method filename is considered the last step in the Schedule, so subsequent steps will not be saved. If your Schedule requires more than 99 injections, create as many new Schedules as necessary and then link them so that they run sequentially. **Do not enter any information on line 100 of the Schedule worksheet; line 100 is reserved for Schedule linking information.** For more information, see *Schedule Editor/Edit Menu/Schedule Linking*.

While **editing** a Schedule means to change the existing information in a cell in the worksheet, and **entering** means to add information to a blank cell, the procedure for accomplishing them is identical:

Point and click inside the desired cell, or use the [Tab] key or keyboard cursor control keys to move from one cell to the next. As you type in information, the

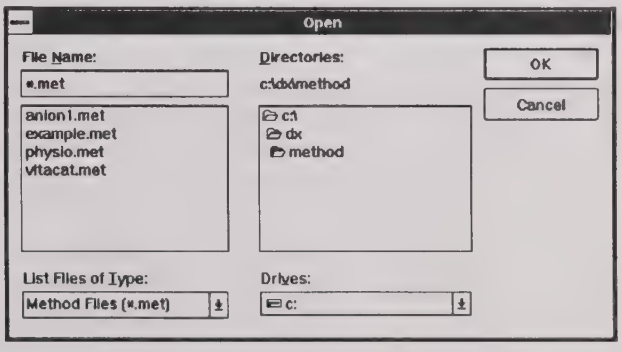


characters will appear in a text editing field above the Schedule worksheet and the status bar at the bottom of the screen will change to "Edit". If the information displayed in the text editing field is acceptable, press [Enter] or [Tab]. The new information will be transferred to the highlighted cell in the table and the message in the status bar will be updated to "Ready".

NOTE: Pressing [Tab] also moves the cursor to the next cell; if the cursor was in the last cell of a line, it will move to the first cell of the next line. Pressing [Enter] does not move the cursor.

Sample Name Type in a name (up to 30 characters) to identify the sample. The special sample name AUTOCALx, where x is the calibration level, causes the Run program to use sample data to calculate the response factors for each sample component and update the Method. The heights, areas, and retention times for each peak are also updated. Use the sample name AUTOCALxR to have the Method updated by replacing (R) the previously stored values with the current values. Use AUTOCALxA to have the Method updated by averaging (A) the new values with the previous ones.

Method Double-click on an empty Method cell to open this dialog box:



Type in the path and filename of the Method you want to use to analyze the sample or, if the Method file already exists, click on the filename in the list of available Method files to enter it in the empty Method cell. (The Method must be stored on disk before you actually execute it as part of a Schedule.)

Schedule Linking

After creating a Schedule, double-click on the first empty Method cell in the worksheet. When the Open dialog box appears, change to the Schedule directory. Change the file type to .SCH and select the desired Schedule name from the directory to have the Schedule copied into the empty Method cell. This Schedule will run immediately after the Schedule currently displayed in the worksheet.

If you want to link another Schedule, edit the last step of this additional Schedule in the same way as above. The number of Schedules that can be linked is limited only by your disk capacity.

Data File Type in a filename of no more than eight characters under which the raw data from the analysis will be stored on disk. **This name takes precedence over the name specified in the Method.**

A filename extension will not be accepted. If you attempt to add an extension, it will be ignored. The extension ".Dxx", where xx is a number from 01 to 99 corresponding to the line number in the Schedule, is automatically added to the filename when the data is stored on disk. The ASCII report file, if requested, is stored under the same data filename but with the extension .Rxx.

If you do not include the path in the filename, the raw data will be stored in the \DATA subdirectory in the directory that holds your AI-450 files. If you do include the path, the Run program will use that path as the default, rather than \DATA. In either case, you can still modify the path when Run loads the Schedule. It is advisable to use a new subdirectory for each new Schedule; this avoids putting too many files in one directory, which reduces computer performance.

The Schedule Editor will automatically create a new filename if any of the following situations occurs:

- A raw data filename that does not have exactly seven characters will be changed.
- A raw data file that is run repeatedly without changing the filename will generate new filenames each time the Schedule is run.
- A Schedule that specifies a Method which uses two detectors will generate a new filename for the second detector.

NOTE: Refer to the section entitled *File Naming* for examples of how to correctly name files.

Vol. If desired, enter the volume of sample injected so that the calculated concentrations will be corrected for differences in sample volumes. If you are injecting the same sample volume each time, use the value 1. This parameter applies only to Methods using external standard calibration.

Dil. The dilution factor provides a mathematical correction (diluent volume/sample volume) for dilution effects. For a dilution of 1:10, enter 10. If there was no dilution, use the value 1. This parameter is ignored by calibration samples.

Int. Std. This value is used in internal standard calculations only. If there is no variation, use the value 1. If there is a variation, enter the value. This value will be compared to the one in the Component Table.

Status Bar displays the current editing status: "Edit", if new information is being entered into a cell, or "Ready", if editing is not in progress. When a command from one of the three Schedule menus is selected, the status bar displays a brief description of the selected command.

File Naming

Seven-Character Filename and Repeated Runs of the Same Schedule

The following rule is followed when the program creates new filenames: the basic filename must contain seven characters. If more than seven characters are entered, only the first seven are used. If fewer than seven characters are entered, trailing zeros are used to fill as required. An eighth character is reserved for the detector number (1 or 2). If this new filename exists, the quantity 1 is added to the seventh character and an attempt is made to open the file. This continues until a filename is created that does not already exist.

Example of File Naming for a Single-Detector Method

For example, if "RAIN" is specified in every line (injection) of a 10-line Schedule to be run each day, the following raw data files are automatically generated on day #1:

RAIN0001.D01

.

.

RAIN0001.D10

If the same Schedule is run again, the original filename still exists and the following set of new filenames is generated on day #2, day #3, etc.:

RAIN0011.D01

.

.

RAIN0011.D10

.

.

RAIN0021.D01

.

.

RAIN0021.D10

etc.

While this may be manageable, this much data can quickly become confusing. An additional problem can arise if it is necessary to abort a run in mid-Schedule. Assume that, due to some problem, Run is ended during the fifth injection of day #3. At that time, we have the following files stored:

RAIN0021.D01

.

.

RAIN0021.D05

When the problem that ended the run is corrected and the Schedule re-run, the filenames change again. The filenames are now as follows:

RAIN0031.D01

.

.

RAIN0031.D05

The sixth injection is named RAIN0021.D06 instead of RAIN0031.D06, as intended.

To avoid this situation, indicate on the Load ACI Schedule screen (see *Run/Load Schedule*) that you wish to run the Schedule again, starting from line 6. Or, change the filename in the Schedule Editor program each time you run the same Schedule.

We recommend that you change the filenames specified in your Schedule each day/week/month (depending on the number of runs) to use filenames such as RAIN123 (for Jan 23) to make it easier to identify the files later. Note that the filename specified in the Schedule takes precedence over the filename specified in the Method. The daily filenames might then run as follows:

Day #1 -
RAIN1231.D01
.
.
RAIN1231.D10

Day #2 -
RAIN1241.D01
.
.
RAIN1241.D10

Day #3 -
RAIN1251.D01
.
.
RAIN1251.D10
etc.

Keep in mind that, if the filename is originally seven characters long and the program is allowed to generate new filenames, the seventh character will be changed to a "0", then a "1", and so forth, which could lead to confusion if the last character is significant. This is a safety feature is intended to avoid overwriting, not to eliminate your responsibility for choosing appropriate raw data filenames.

Examples of File Naming for a Dual-Detector Method

If a Method specifies two detectors, the eighth character uses 1 for the first detector data and 2 for the second detector data. In the example above, files generated each day might appear as follows:

Detector 1	**Detector 2**
Day 1 -	
RAIN1231.D01	RAIN1232.D01
.	.
.	.
RAIN1231.D10	RAIN1232.D10

Day 2 -

RAIN1241.D01	RAIN1242.D01
.	.
.	.
RAIN1241.D10	RAIN1242.D10

Day 3 -

RAIN1251.D01	RAIN1252.D01
.	.
.	.
RAIN1251.D10	RAIN1252.D10

etc.

Careful planning is mandatory when a large number of dual-detector runs are to be done.

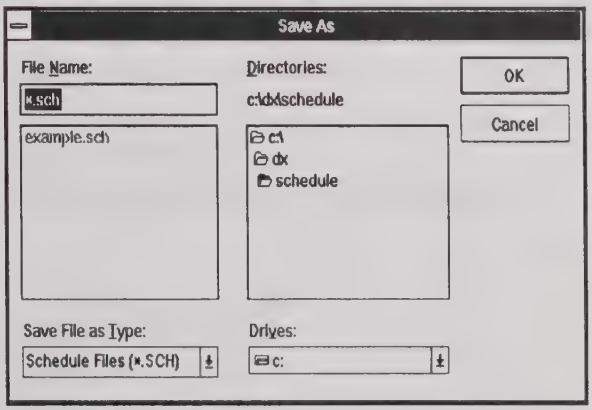
Even if your Method specifies that raw data is not to be stored, you can still store the raw data by specifying the data filename in the Schedule.

Save

If the Schedule was previously saved, selecting Save rewrites it to the disk under the same name as before. If the Schedule was not previously saved, Save functions in the same way as the Save As... command.

Save As...

Save As... opens the Save As dialog box so that you can store on disk the Schedule file being edited:



Type a Schedule filename of up to eight characters (letters and/or numbers, no punctuation or spaces) in the dialog box. If the filename is already present in the specified directory, a warning will ask for verification that you want to replace the existing file.

The Schedule Editor will automatically add the subdirectory \SCHEDULE and the extension ".SCH" to the filename to identify it as a Schedule file. You can enter a different path as part of the filename, if desired. The filename will be added to the \SCHEDULE directory.

Print...

Prints the information from the Schedule currently displayed, as in this sample printout:

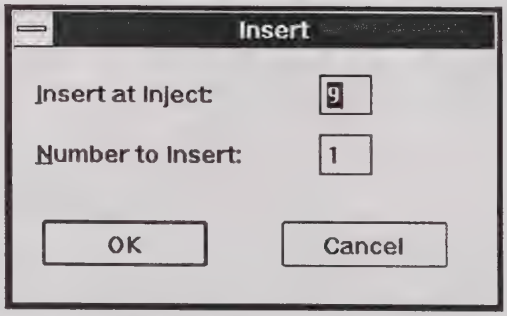
DIONEX SCHEDULE - F:\PUBS\DX\SCHEDULE\EXAMPLE.SCH

Inj#	Sample Name	Method	Data File	Vol.	Dil.	Int.Std.
1	AutoCall	..\example2	..\example2	1	1	1
2	lemon-lime soda	..\example2	..\example2	3.5	1	1
3	lemon-lime concentrate	..\example2	..\example2	1	1	1
4	grape juice Lot 2267	..\example2	..\example2	1	10	1
5	autocall	..\physio.m	..\physio	1	1	1
6	lemon-lime sode	..\physio.m	..\physio	3.5	1	1
7	lemon-llime concentrate	..\physio.m	..\physio	1	1	1
8	grape juice Lot 2267	..\physio.m	..\physio	1	20	1

Edit Menu

Insert...

Inserts one or more blank lines between two existing injections. The injection already at the specified line, as well as all remaining injections, will be renumbered.

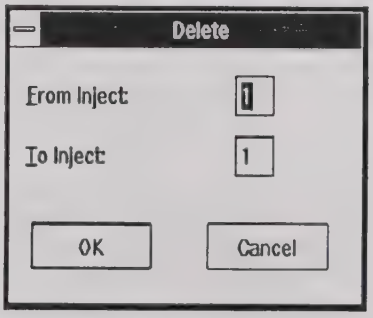


Insert at Inject specifies the line at which the inserted line will appear.

Number to Insert specifies the number of blank lines to be inserted.

Delete...

Deletes one or more injections from an existing Schedule. All lines between, and including, the first and last lines specified will be deleted.

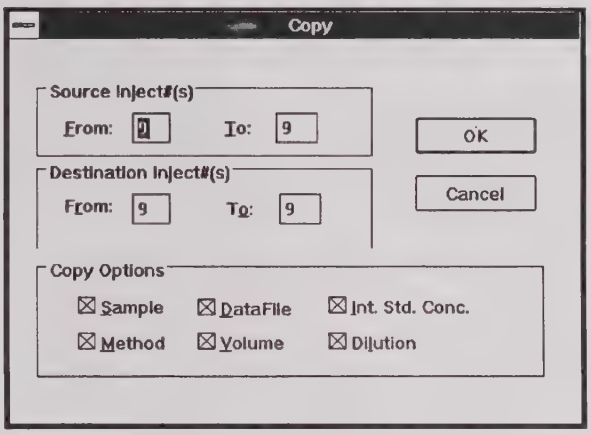


From Inject specifies the first line to be deleted.

To Inject specifies the last line to be deleted.

Copy...

Copies one or more lines of a Schedule to another location in the Schedule. Any lines already present at the second location will be lost, so be sure to insert enough blank lines to prevent them from being overwritten.



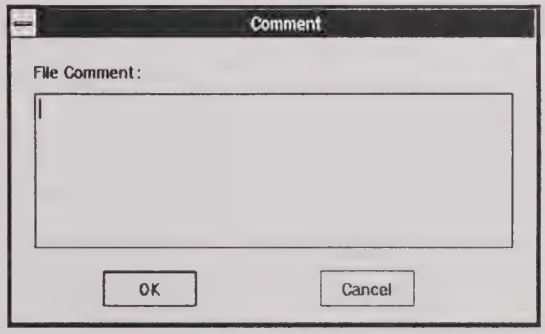
From Injects #(s) specifies the first and last lines of the block to be copied. Setting the first and last lines to the same value copies just the line at that position.

To Injects #(s) specifies the position that the block is to be copied to. The number of lines specified here must be an even multiple of the number of lines specified in "From Inject #(s)". By specifying a "To Inject" range that is larger than the number specified in the "From Inject" range, you can fill a Schedule with repeated blocks of the specified lines.

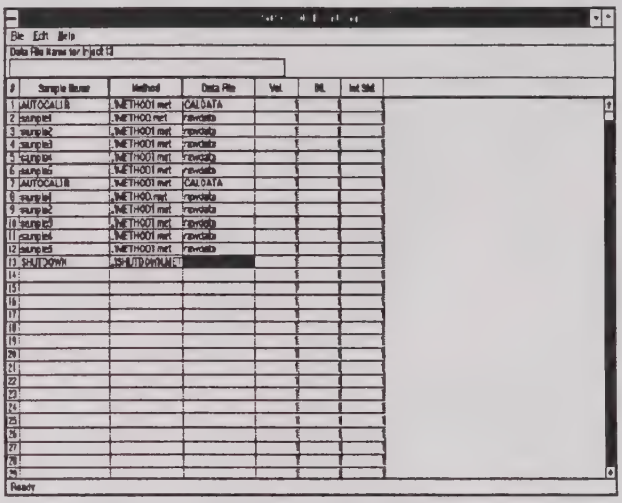
Copy Options allows you to specify which Schedule cells you want copied. This allows you to copy only those cells that are common to each of the steps.

Comment...

Enter any desired remarks (up to 255 characters) about the Schedule in this dialog box. The comments are saved as part of the file.



Example: Creating a Schedule



This example Schedule will calibrate the system, analyze five samples, repeat the entire process, and then shut down.

1. Using the mouse, point and click on the Sample Name cell. Type in the sample name AUTOCAL1R. Press the [Tab] key to simultaneously transfer AUTOCAL1R to the Sample Name cell and move the cursor to the Method cell.
2. Type in the Method name METHOD1. Press the [Tab] key to enter this in the Method cell and move the cursor to the Data File cell.
3. Type in the filename CALDATA and press [Tab].
4. Tab through the Volume, Dilution, and Internal Standard cells, leaving the default values of 1 unchanged.
5. For Step 2 of the Schedule, change the sample name to sample1 and the name of the data file to "rawdata". Press [Tab] to enter the new names in the appropriate cells.
6. Repeat Step 5 four more times, changing the sample name for each injection.
7. Select the Copy command from the Edit menu. Specify lines "From 1 To 6" as the Source Injects #(s).
8. Specify lines "From 7 To 12" as the Destination Injects #(s).
9. Select all of the Copy Options.
10. Press OK. Note that the information in lines 1-6 is copied into lines 7-12.
11. On line 13, type SHUTDOWN in the Sample Name cell. Tab over to the Method cell and type SHUTDOWN.MET. Press [Enter] or [Tab].

The Run program conducts the analyses specified by your Methods and/or Schedules. It loads data acquisition and control instructions into the Advanced Computer Interfaces(s), monitors the status of each system, and processes chromatograms according to the data processing parameters selected for the Method.

The initial (INIT) conditions in the Timed Events sub-file of the Method take effect as soon as the Method is loaded into Advanced Computer Interface (ACI) memory. If a gradient program file was transferred to the gradient pump, the gradient program is copied into the pump's run memory. If the initial conditions include an instruction to start the gradient pump motor, the pump begins operating according to the time zero step of the gradient program. The initial conditions remain in effect until the analysis is started, allowing the system time to stabilize.

You can start an analysis via the Start command in the Run program, the RUN button on the ACI front panel, or the RUN input relays on the ACI rear panel. The ACI timed events clock starts when the analysis starts; data collection begins when the Start Sampling event occurs.

Raw data from the detectors is stored in ACI memory until retrieved by the computer. The computer uses the data processing parameters in the Method to process the raw data, store it on disk, and then prepare a report of the results.

As you know, a Schedule not only allows unattended analysis of multiple samples, but makes it possible to analyze each injection using a different Method and sample name, if desired. When a Schedule is run, only one line at a time is downloaded to the interface. After each line in the Schedule is run, the Run program automatically loads the next line. The raw data used for each sample, as well as a copy of all the Method parameters, are stored under the data filename specified in the Schedule.

Implementing the Schedule linking feature allows almost unlimited automated sample analysis (see *Schedule Editor/Schedule Editor Worksheet/Schedule Linking*). The Run program checks each Method file in a Schedule for the .SCH extension. If the file is a Schedule, Run will use it for subsequent injections.

Considerations When Using a Gradient Pump

Although you can load gradient programs into the gradient pump while it is in either LOCAL or REMOTE mode, we recommend doing so only in REMOTE. If you load a program while the pump is in LOCAL, the initial control conditions are not executed.

NOTE: You must switch to REMOTE before issuing a Start command from the Run program.

Until you issue the Start command, the gradient pump will continue operating according to the parameters it was using while in LOCAL. Only then do timed events commands from the interface control the pump. Under these conditions, the INIT step of the timed events sub-file must include instructions to start the pump, reset the gradient clock, and hold. One step to hold the gradient clock at initial condition and a second step to run the gradient clock at T = 0.0 must be included to copy the gradient program into the pump's run memory, where it controls the gradient.

Starting the Run Program

To start the Run program, double-click on the Run icon in the AI-450 MainMenu or on RUN.EXE in the directory that holds your AI-450 files. The main window is initially blank, with six pull-down menus on the menu bar. The commands, dialog boxes, and windows associated with each menu are described in the following pages.

RUN.EXE Command Summary

Monitor Menu
- ACI SYS1 or SYS2
- Polling Rate...
- Printer...
- Close
- Exit

Load Menu
- Method...
- Schedule...

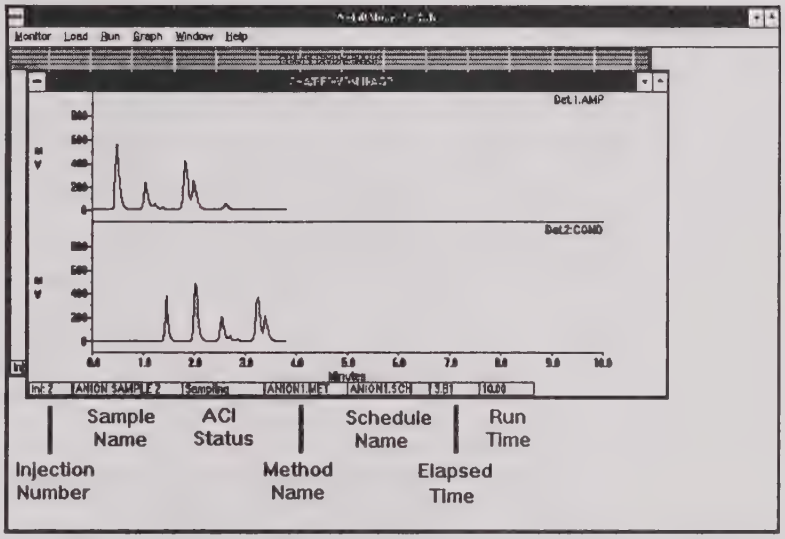
Run Menu
- Start...
- End...
- Abort...

Graph Menu
- Scale...
- Superimpose Plots
- Reverse Background
- Grid Lines

Window Menu
- Tile
- Cascade
- Arrange Icons
- Close All
- Select Active Systems

The AutoMonitor Polling Window

The Run program can monitor multiple systems simultaneously. Each active system has a separate child window, with the system name shown in the title bar. To set the focus to a system's child window, click on the window or select the system name from the Window menu. When an Advanced Computer Interface (ACI) starts sampling for a system, the real-time chromatogram is displayed in the window and the system status is shown in the status bar at the bottom of the window.



Injection Number indicates which Schedule line or injection is running. If a Schedule is not being used, Inj indicates the number of times the same Method has been run since it was last downloaded.

Sample Name displays the name associated with the sample currently running. The current sample name can be changed through the Load Method or Load Schedule commands.

ACI Status indicates the status of each system:

MESSAGE	STATUS
NO ACI	No powered-up ACI is connected.
NOT LOADED	The ACI/System is not currently programmed with a Method.
READY	The ACI/System has been programmed with a Method and is ready to begin an analysis.
RUNNING T.E.	The ACI has started executing the timed events in the Method, but is not yet collecting data.
SAMPLING	Data is being collected from the designated system, and the partial chromatogram can be uploaded to the computer.

MESSAGE	STATUS
ACQ.DONE	Data collection is finished; the complete chromatogram is stored in ACI memory and is ready for processing and storage by the computer.
ALARM ON	A pressure limit alarm has stopped the pump. After clearing, the alarm status will change to READY. The Method can then be rerun or a new one downloaded.
ACI FULL	ACI memory is full, and the raw data has not been uploaded by the computer. The ACI stopped collecting data before the end of the run time specified in the Method.
WRAPPED	ACI memory is full; some raw data from the beginning of the chromatogram has been uploaded, and the memory used for the early part of the chromatogram is being overwritten.

WARNING: If you see the WRAPPED message, do not close the child window or data will be lost.

Method Name indicates the Method in use.

Schedule Name indicates the Schedule in use, if any.

Elapsed Time indicates how much time has elapsed since data collection began.

Run Time indicates the total period of time during which the current Method will collect data. This time is specified by the Run Time parameter in the Method.

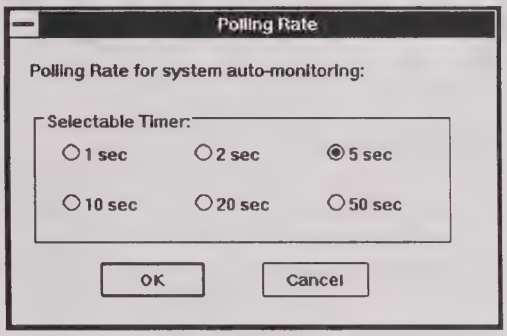
Monitor Menu

ACI SYS1 or SYS2

Open the window for system 1 or 2, or both, by selecting the appropriate command(s). (If a window is already open, the option is grayed out.)

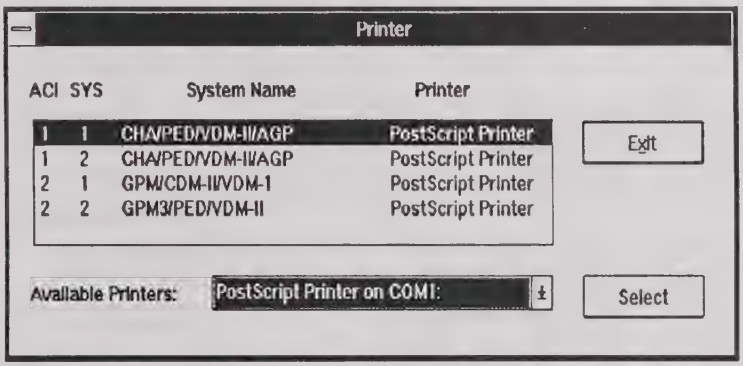
Polling Rate...

The polling rate determines how often the computer checks the ACI status and retrieves data. The selected polling rate applies to all systems. The polling rate is saved in the AI450.INI file, so each time you start the Run program, the polling rate will still be set to the rate last selected.



Printer...

Use the controls in the Printer dialog box to make the ACI printer assignments. Each system of an ACI can be assigned to a different printer, if desired, and if other printers are installed. The name of the default printer (i.e., the printer installed in Windows Control Panel) is displayed at the top of the Available Printers drop-down combo box.



First click on the ACI/System name, to highlight it. If you want to assign the default printer to this ACI/System, simply click on the default printer name and then on the Select command button. To select a different printer, click on the arrow beside the drop-down combo box to display your options. Click on the desired printer name and then on Select. When you finish selecting printers, click on Exit to close the dialog box.

Close

Closes the highlighted system window and system. Any plot information on the screen at the time is lost, and polling stops for that system. The interface, however, will continue to run the analysis and collect data.

Exit

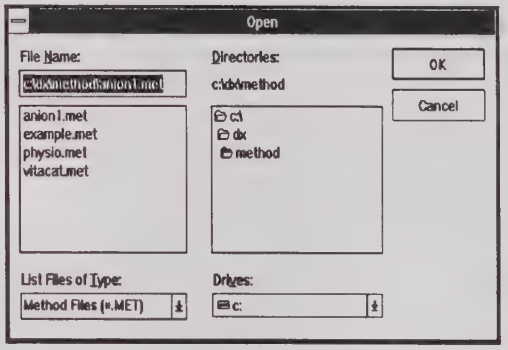
Closes the Run program.

Load Menu

Before selecting one of the commands from the Load menu, verify that all connections between the chromatograph components and the ACI are complete; also verifythat the ACI is powered up and is connected to the computer. If you want the initial conditions to control your system during equilibration, set all modules to REMOTE.

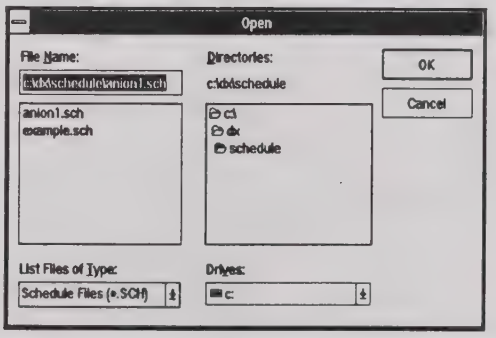
NOTE: The system status must be either NOT LOADED or READY before you can load a Method or Schedule.

Load Method...



Specify the path and filename of the Method you want to use for the selected system from this dialog box. The Method file will be retrieved from disk and loaded into the ACI. If the Method includes a gradient, the gradient program will be loaded into the gradient pump. If the selected system does not match the system assignment stored in the Method, a message box will suggest what to do.

Load Schedule...

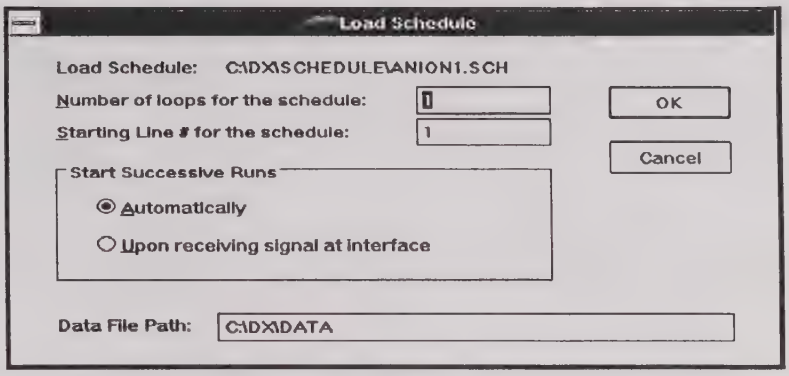


Specify the Schedule you want to use to analyze a series of samples from this dialog box. If the system assignment of the first Method does not match the system you are attempting to load, a message box will suggest what to do.

NOTE: The file types listed depend on the type of autosampler configured:

AUTOSAMPLER	FILE TYPE
None	*.SCH
Spectra-Physics SP8880 Autosampler	*.ASC
Spectra-Physics AS3500 Autosampler	*.AS3

Before the Schedule is loaded, another dialog box will prompt you for the conditions under which the Schedule is to be run:



Number of loops for the Schedule is the number of times the Schedule is repeated (from 1 to 99). If you want the Schedule to run continuously, enter -1. (This option is grayed out when an SP8880 or AS3500 Autosampler is configured.)

Starting Line # for the Schedule is the Schedule line number at which analyses begin. This option lets you start, or resume with, any line number. Each time raw data is stored, the extension of the data filename is indexed to match the injection number, starting with the number entered here. (This option is grayed out when an SP8880 Autosampler is configured.)

Start Successive Runs determines how the next analysis in the Schedule is started.

Select **Automatically** if you want the timed events for the next Method in the Schedule to start automatically as soon as the data reduction process from the preceding analysis is completed.

When you select **Upon receiving signal at interface**, the ACI does not start the timed events from the next Method until it receives a signal from the front panel or from a remote device. This makes it possible for your autosampler to signal the computer that the next sample has been loaded and injected and is ready for analysis.

Data File Path The data file path associated with the Schedule automatically appears, but can be edited. If no file path is associate with the Schedule, the program automatically creates one.

Run Menu

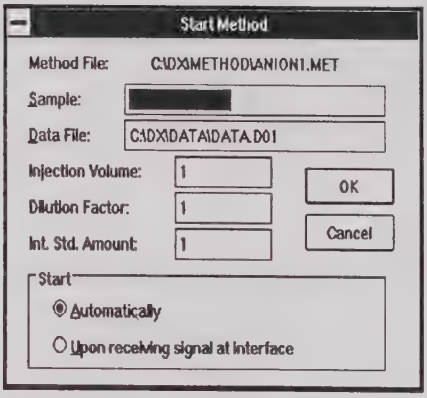
Start...

This signals the ACI to begin executing the timed events of the currently loaded Method. As soon as the ACI executes a Start Sampling timed events for a system, the system window displays the real-time plot of the data as it is collected. The timed events can also be started using the RUN button on the ACI front panel, or by receiving a signal at the RUN relay contacts on the ACI rear panel.

NOTES:

1. A Method can be run repeatedly, using the Start command, without downloading at the end of each run.
2. Because of the way raw data is collected and stored by the ACI, both detectors of a dual-detector system must operate at the same data sampling rate.

When you select Start..., a dialog box with the default parameters from the loaded Method file appears. This dialog box allows you to modify the default parameters without having to reload the Method. Any changes made here will be saved in the raw data file.



Sample Name Type in a name (of no more than 30 characters) to identify the sample.

Use the special sample name AUTOCALx, where x is the calibration level (1-10), only if the sample is the calibration standard. AUTOCALx causes Run to use the current peak areas and heights to update the Method Component Table. Entering an *R* or an *A* after the calibration level in the AUTOCALx sample name specifies that the Method be updated either by replacing (R) the previously stored values with the current values or by averaging (A) the two values.

Data File To use a filename other than the one specified in the Method, enter a new name of from one to seven characters. If the new name has fewer than seven characters, the Run program adds zeros to obtain a total of seven characters. It also adds as an eighth character either a 1 or a 2; 1 indicates the first detector data file, and 2 indicates the second detector data file.

Unless you enter a different directory and pathname before the filename, the data will be stored in the directory you chose for your AI-450 files. The data filename specified in the Method is not changed. The extension .Dxx, where xx is a number from 01 to 99, indicates the iteration since the last download. It is automatically added to the filename when the data is stored on disk.

Injection Volume Enter the volume of sample injected (using the same unit as the calibration standards), so that the calculated amount can be corrected for differences in sample volumes. (This parameter is used only in external standard calculations.)

Dilution Factor provides a mathematical correction for dilution effects. For example, for a dilution of 1 to 10, enter 10. If there was no dilution, use the value 1. (This parameter is used by external and internal standards and is ignored by calibration samples.)

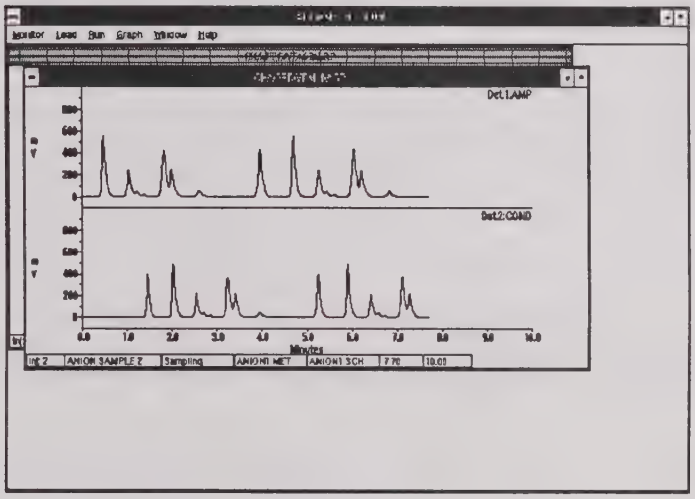
Int. Std. Amount Enter the amount of the internal standard (if one is used) so that the calculated amount can be corrected for differences in the internal standard amount. (If you are not using an external calibration standard, this parameter will be ignored.)

Start determines how the next Method is started.

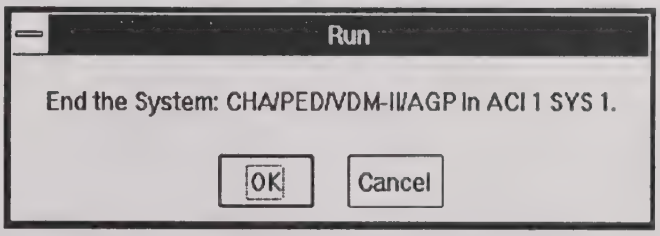
Select **Automatically** if you want the timed events for the loaded Method to start immediately.

When you select **Upon receiving signal at interface**, the ACI does not start the timed events from the loaded Method until it receives a signal from the front panel or from a remote device. This makes it possible for an instrument (e.g., your autosampler) to signal the ACI that the next sample has been loaded and injected and is ready for analysis.

In a dual-detector system, the real-time plots for the detectors are displayed in the window, either one above the other (as shown below) or superimposed. The type of display depends upon the option chosen from the Graph menu (see *Run/Graph menu*).



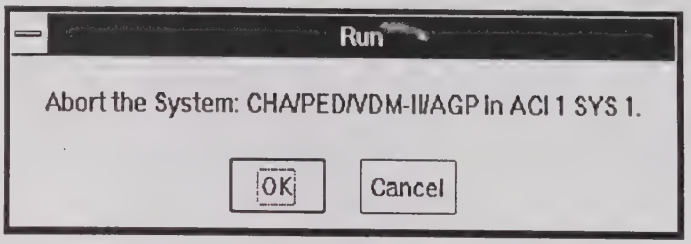
End...



Use End... to finish a run early, but keep the data. End... terminates execution of the Method **and** Schedule for the specified ACI/System. The remaining timed events are executed immediately, leaving the system operating under the conditions specified in the last step of the Timed Events file. (The END button on the ACI front panel and the END contacts on the ACI rear panel perform the same function.) If the Method that was being run so specifies, the result report for the analysis up to the point of interruption will be printed and the raw data will be saved.

NOTE: Unless the last step of the Method Timed Events file includes a Reset command (see *Method Editor/Settings Menu/Control*), the gradient pump will continue to operate according to the last step in the gradient program.

Abort...



Use the Abort command to finish a run early and discard the data. Abort terminates execution of the Method **and** Schedule for the specified ACI/System. The remaining timed events are executed immediately, leaving the system operating under the conditions specified in the last step of the Timed Events file. If this command is selected for an active system, an Abort dialog box appears; if the system is not active, a Not Sampling box appears. Unless you include a Reset command in the last step of the Method Timed Events file (see *Method Editor/Settings Menu/Control*), the gradient pump will continue to operate according to the last step in the gradient program.

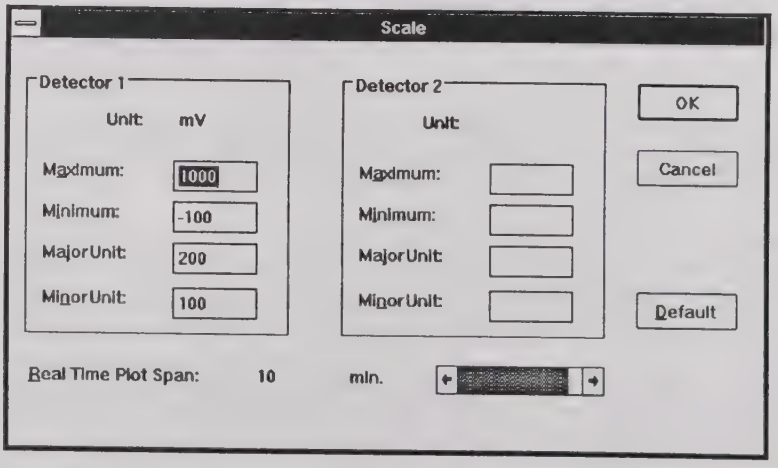
NOTES:

1. After aborting a run, wait at least 10 seconds before restarting **from the front panel.** You do **not** need to wait to restart from the computer screen.
2. The timed events can also be aborted using the ABORT button on the ACI front panel.

Graph Menu

Commands on the Graph menu control the scaling of the real-time plot. These commands are only active during real-time data acquisition (i.e., when the Status is Sampling).

Scale...



Changes the maximum and minimum points and the major and minor divisions for the Y axis of the real-time plot for each detector. You can also change the length of the time axis for the real-time plot for each detector. This command changes the plotting parameters of the active system only.

Unit indicates the units for the Y axis of the plot specified in the Method.

Maximum Enter the maximum value, in the specified units, for the Y axis of the plot.

Minimum Enter the minimum value, in the specified units, for the Y axis of the plot.

Major Unit Enter the number of units represented by each major division on the Y axis.

Minor Unit Enter the number of units represented by each minor division on the Y axis.

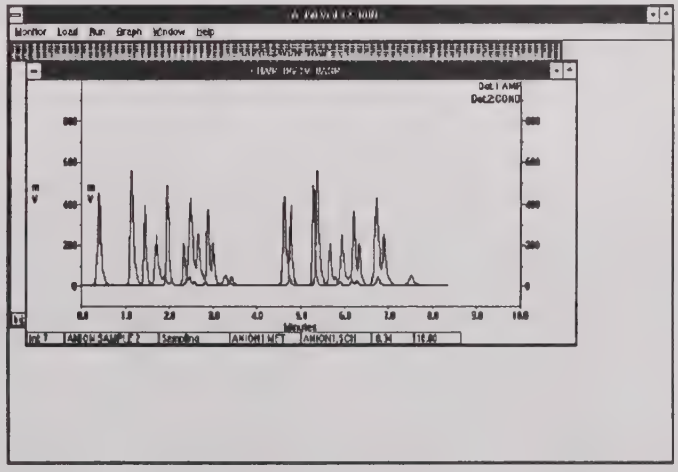
Default sets the Y axis to the values specified in the Method.

Real-Time Plot Span Use the scroll bar to specify, in minutes, the real-time plot span. As the plot reaches the end of the window, the window shifts over automatically.

Superimpose Plots

Allows you to view real-time plots in either a superimposed format (as in the screen below) or as vertically separated plots on separate axes. Clicking on this command selects the superimposed format; clicking on it again returns the screen to the separated-plot format.

NOTE: This option is available only for dual-detector systems.



Reverse Background

Selects white or black as the background color of the plot display.

Grid Lines

Superimposes grid lines on the polling window.

Window Menu

Tile

Resizes and positions all open windows so that each is fully visible.

Cascade

Arranges windows in an overlapping fashion so that each title bar is visible.

Arrange Icons

Automatically arranges icons neatly on the screen.

Close All

Closes all active windows.

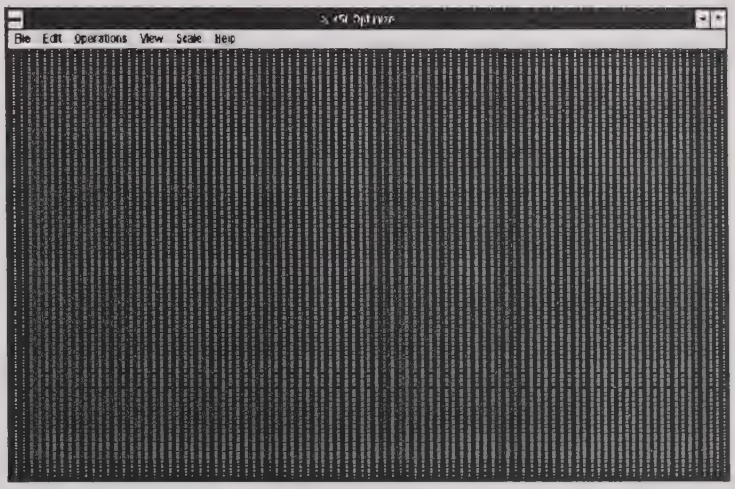
What Is Optimize?

The Optimize program is used to explore the details of a chromatogram; fine-tune the integration; assign peak names; and update Method files and data files to include new parameters. You can print out reports and chromatograms or copy the information to the Windows Clipboard, from where it can be copied to other software programs.

Optimize creates and uses the following files:

Files Used	Files Created	Comments
*.ME?		Methods
*.GPM		Gradients (for Gradient Overlay)
*.PRF		Printed Report Format files
*.D??	*.D??	Raw data files
*.DAT	*.DAT	DataMath raw data files
	*.R??	Result files
	*.CSV	File exported to Excel (or other program)
	*.PRN	File exported to Lotus 1-2-3 (or other program)
	*.TE	.TE, .GPM, and .PED files are
	*.GPM	imbedded in raw data files and
	*.PED	can be extracted later.

Starting the Optimize Program



To start the Optimize program, double-click on the Optimize icon in the AI-450 MainMenu or on OPTIMIZE.EXE in the directory that contains your AI-450 files. The Optimize main window will appear. There are six pull-down menus. The commands, dialog boxes, and additional windows associated with each menu are shown and described on the following pages.

OPTIMIZE.EXE Command Summary

File Menu
- Open Data File...
- Open Method File...
- Save File As...
  - Raw Data
  - Method
- Export...
- Printer Setup...
- Print...
- View Report...

Edit Menu
- Integration...
- Data Events...
- Calibration...
- Components...
- Report...
- Copy...
  - Graphics
  - Report

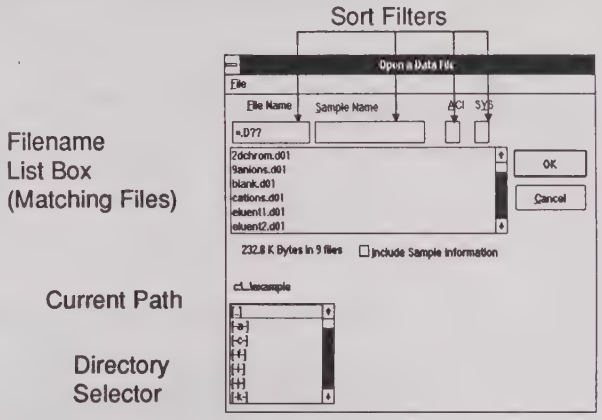
Operations Menu
- Auto Threshold
- Adjust Integration...
- Set Baselines Manually...
- Name Peaks...
- Calibrate...

View Menu
- Reference Plot
- Grid
- Gradient Display...
- Pen Size...
- Baselines
- Start/Stop Markers
- Peak Fills
- Peak Labels...
- Fonts...

Scale Menu
- Undo
- Scales...
- Full View
- AutoScale
- Zoom
  - Zoom In Vertically
  - Zoom Out Vertically
  - Zoom In Horizontally
  - Zoom Out Horizontally
- Scale Tools

File Menu

Open Data File...



Select Directory Select a directory by clicking on the applicable disk drive and directory in the Directory Selector box.

Sort Filters simplify your search for a specific data file by filtering out all files which do not match the criteria you enter into the Filename, Sample Name (if selected), and Interface/System Sort Filters (if selected).

Filename Type a filename of one to eight characters (no punctuation or spaces) and the extension .D??, where ?? is a number from 01 to 99 and corresponds to the inject number on the schedule.

Sample Name This filter can be used only when the Sample Name Box is active. Type a sample name of one to eighteen characters (no punctuation or spaces).

ACI/SYS Depending on which data system model you are using, select an Interface, 1 through 6, and System 1 or 2. If you leave these boxes blank, selected files for all interfaces and systems are displayed.

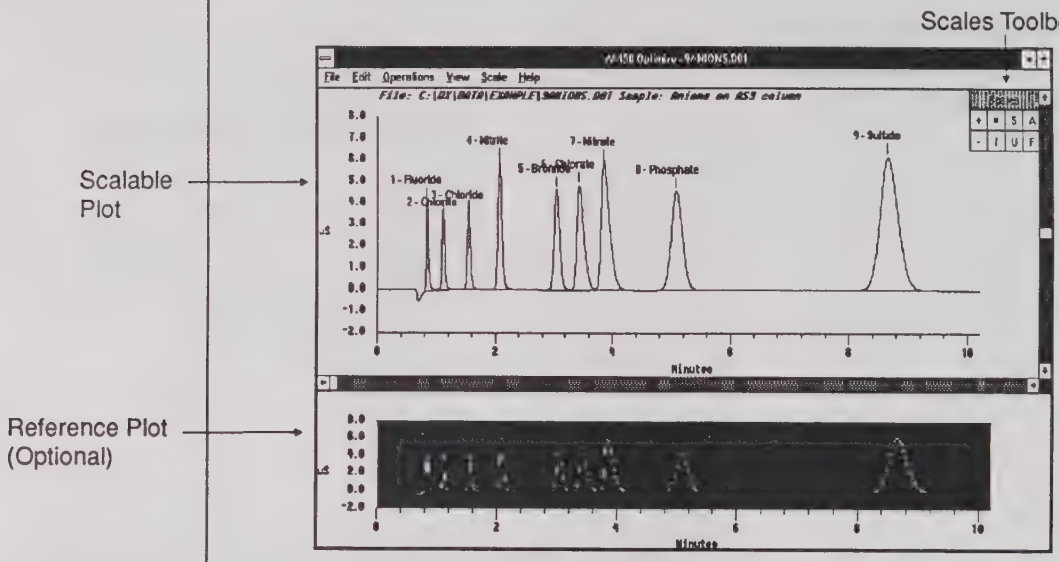
Wild Cards are two special characters, ? and *, that can be used when entering Sort Filters. The ? indicates that any character can occupy that position. The * indicates that any character can occupy that position and all remaining positions in the name, extension, or number field being entered. Wild Cards **cannot** be used with Sample Names.

Include Sample Information Click on the check box to include or exclude sample information for the files. Directory searches are much faster if sample information is not included.

OK opens a highlighted file.

Cancel cancels the Open a Data File operation.

Select File Select the file you want to open by clicking on the name of the file in the Filename list box. When you do, a screen similar to this one will be displayed:



The **scalable plot** is displayed in the editing window.

The **reference plot** is the complete chromatogram, with a highlighted area indicating the view shown in the editing window. In the screen shown above, the entire reference plot is highlighted because the full chromatogram is displayed in the editing window. You can show or hide the reference plot by clicking on the Reference Plot option (see *Optimize/View Menu*).

The **Scales Toolbox** contains symbols that represent various graphic controls that you can use to alter the view of the scalable plot. To select a control, position the mouse cursor on the desired symbol and click the mouse left button. These graphic controls can also be selected from the Scale menu, but the toolbox allows you to access them more quickly. You can show or hide the toolbox by clicking on the Scale Tools option (see *Optimize/Scale Menu*).

+ (Zoom In Vertically) increases the distance between the Response Axis scale ticks, thereby showing a narrower section of the chromatogram at greater vertical magnification.

- (Zoom Out Vertically) decreases the distance between the Response Axis scale ticks, thereby showing a broader section of the chromatogram at less vertical magnification.

*** (Zoom In Horizontally)** increases the distance between the Time Axis scale ticks, thereby showing a narrower section of the chromatogram at greater horizontal magnification.

/ (Zoom Out Horizontally) decreases the distance between the Time Axis scale ticks, thereby showing a broader section of the chromatogram at less horizontal magnification.

S (Scales) opens the Scale Options dialog box, which allows you to set the scales to exact values (see *Optimize/Scale Menu* for details).

A (Autoscale) automatically sets Scale Top to Highest Peak and Scale Bottom to Lowest Value, in order to maximize peak size and contrast.

F (Full View) displays the entire chromatogram in the editing window.

U (Undo) changes the editing plot from its present scale to the previously set scale.

Graphics Controls

This section describes several graphics controls you can use to select viewing options.

Menu Accelerators

Some menu commands can be executed instantaneously via keystrokes:

1. If the name of the option is followed by a caret (^) and a letter, hold down the <Ctrl> (Control) key and press the letter.
2. If the name of the option is followed by the character +, -, *, or /, press the character on the keyboard.

Enlarge a Selected Area of the Chromatogram

Starting at one corner of the area you want enlarged, drag the mouse cursor diagonally across the area. When you release the mouse button, everything within the resultant reverse-contrast rectangle is enlarged and displayed in the editing window. This enlarged view is correctly plotted in reference to the X and Y scales, but may have a different visual symmetry than the original.

Move the Time-Scale Cursor

The Time-Scale cursor is the vertical-line cursor on the chromatogram in the Integration Control, Manual Baseline Editing, and Name Peaks dialog boxes. There are four ways to move the cursor:

1. Position the mouse cursor on the Time-Scale cursor, depress the mouse right button and drag both cursors to the desired location.
2. Place the mouse cursor at the location where you want to position the Time-Scale cursor and depress the mouse right button. The Time-Scale cursor will jump to the desired location.
3. Use the mouse to adjust the scroll bar located above the editing window. This scroll bar lets you move the Time- Scale cursor point-by-point along the time axis. Depressing the <Alt> key while using the scroll bar moves the cursor ten points at a time.
4. Use the Down and the Right Arrow Keys to move the Time-Scale to the right, relative to the cursor. Use the Up and the Left Arrow Keys to move the Time-Scale to the left.

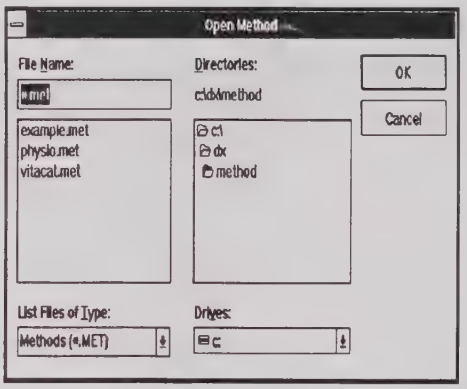
Move the Entire Chromatogram Horizontally or Vertically in the Editing Window

Position the cursor on the scroll bar beneath or at the right side of the chromatogram, then depress and hold the mouse right button to move the editing window.

Stretch the Chromatogram View Horizontally or Vertically

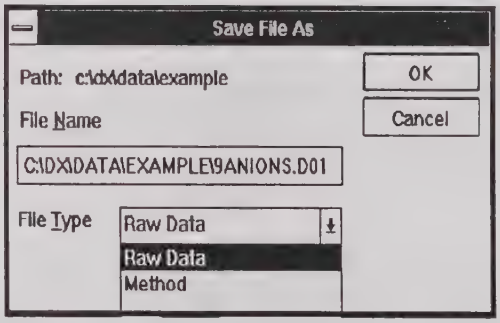
1. Depress the <Shift> key.
2. Position the cursor on the scroll bar beneath or at the right side of the chromatogram.
3. Depress the mouse right button.

Open Method File...



Select a Method to replace in active memory the Method currently being used to interpret open raw data files. These Method parameters are not replaced permanently as part of the raw data file unless the data file is saved.

Save File As...

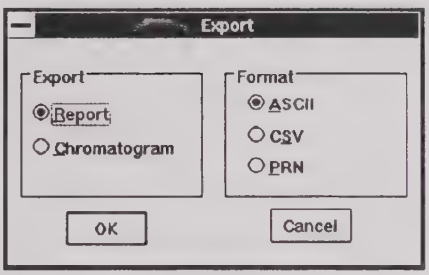


Allows you to select a file type, and then name and save on disk the currently displayed raw data file or Method parameters. All changes to the integration and component identification are saved with the file.

This selection is normally used to do one of the following:

- Save a newly optimized file to disk
- Extract a Method, Timed Events, Gradient, or PED file from a raw data file. Since these files are embedded in raw data files, you can retrieve the original file. This is a convenient way to recover a file that was deleted or was modified since the sample was run.

Export



Exports the current report or raw data values of a chromatogram in a format compatible with other software programs (word processors, spreadsheets, etc.). Unless you specify otherwise, files are saved in the directory containing the original data file.

Export

Report exports the report in the format selected. To review the information in the report, select the View Report option from the File menu.

Chromatogram opens the Chromatogram Export Options dialog box (see below).

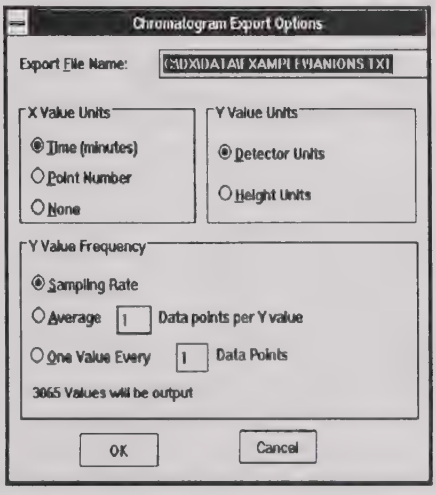
Format

Select **ASCII** when exporting chromatogram files and creating files to be used with word processing programs.

Select **CSV** when creating report files for use with spreadsheet and database programs such as Excel and dBase.

Select **PRN** when creating report files to be used with Lotus programs such as 1-2-3 or Symphony.

Selecting the Chromatogram option opens the Chromatogram Export Options dialog box:



X Unit Values exports raw data values as either minutes or point numbers. You can also elect to omit these values from the report.

Y Value Units exports raw data values either as Detector Units (e.g., AU for an absorbance detector) or Height Units (e.g., microvolts for an analog voltage output reading).

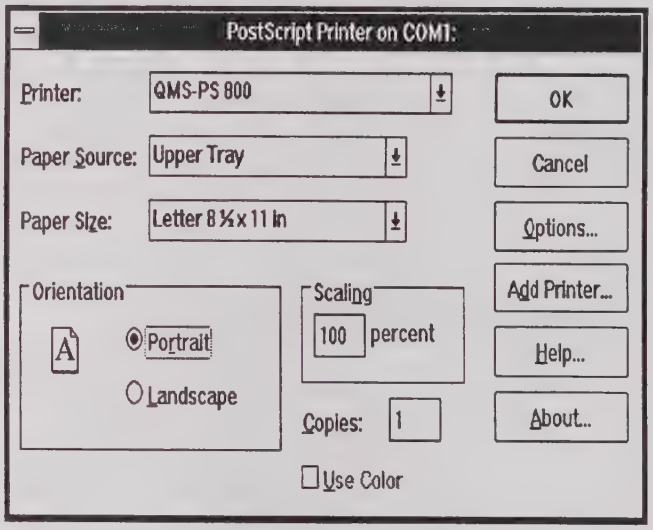
Y Value Frequency controls the number of data points that are exported.

Sampling Rate One data point is exported for each data point in the chromatogram.

Average Each data point exported is the result of averaging the number of data points entered here.

One Value Every One data point is exported per the number of data points entered here. For example, if you entered 10, every tenth data point would be exported.

Printer Setup...

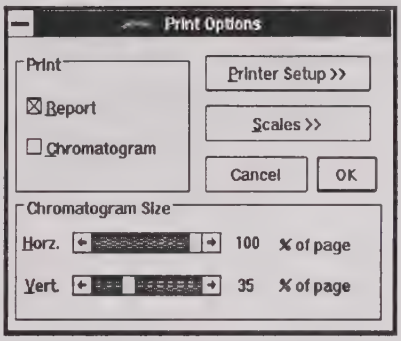


The format and contents of the printer dialog box vary according to the printer(s) installed in Windows Control Panel. Typical options include graphics resolution, paper size, number of copies, orientation, and test mode.

Note that the lower the graphics resolution setting, the faster the printing. The quality of the image produced with the lowest resolution is fine for most printouts, but you should select a higher resolution any time sharper images are required.

For best results when using a plotter, select Draft as the Text mode.

Print...



Print You can print a report, a chromatogram, or both. Printing is much faster if a chromatogram is not included.

Chromatogram Size Use the horizontal and vertical scroll bar to select the size (as a percentage of a page) of the printed chromatogram. The terms horizontal and vertical refer to the orientation of the printed page as you view it. For example, horizontal in relation to portrait orientation indicates the narrow dimension of the paper.

Printer Setup >> opens the same dialog box described previously in Printer Setup....

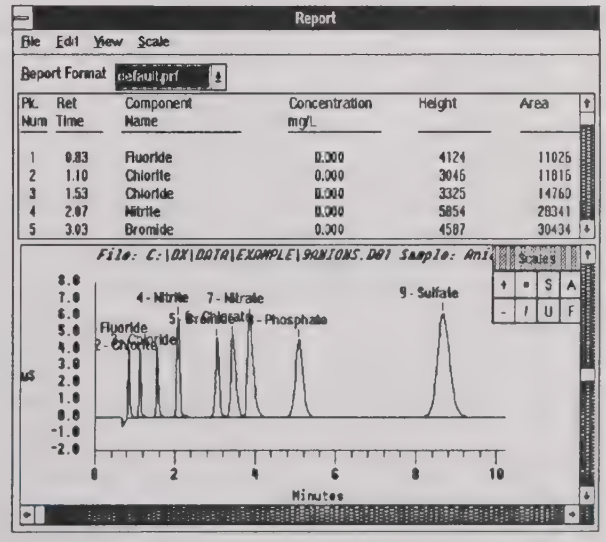
Scales >> opens the Scale Options dialog box (see *Optimize/Scale Menu/Scales....*).

Cancel returns you to Optimize's main window without making any changes.

OK incorporates your selections and sends the report and/or chromatogram to the printer.

View Report

Provides a preview of the peak report so that you can make any final changes before printing. To change the report format, open the Report Format drop-down list box below the menu bar and select a different .PRF file.

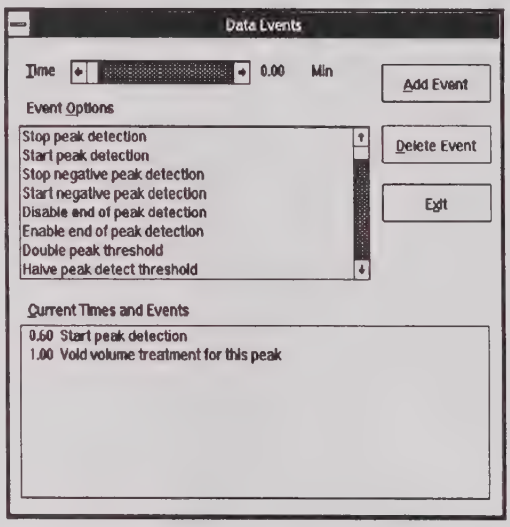


Edit Menu

Integration..., Calibration..., Components..., and Report...

You can edit a Method's Integration, Data Events, Calibration, Component, and Report parameters directly from Optimize, without having to exit the program and open the Method Editor. The screens in Optimize are identical to those in the Data Processing section of Method Editor.

Data Events...



As you use the graphic integration controls, data events are added to your Method. You can edit these data events in the Data Events dialog box.

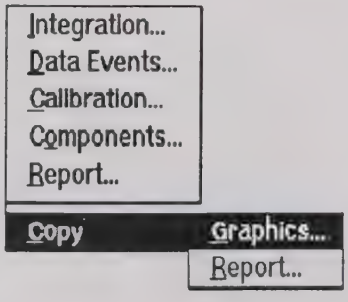
To add an event to the Method, highlight that event in the Event Options list box, use the Time scroll bar to set the time at which the event occurs, and click on the Add Event command button. The new information will be added to the Current Times and Events list box.

To remove an event from the Method, highlight that event in the Current Times and Events list box and then click on the Delete Event command button.

When you finish editing, select Exit to return to the Integration Control dialog box. There, click on the Re-Integrate command button.

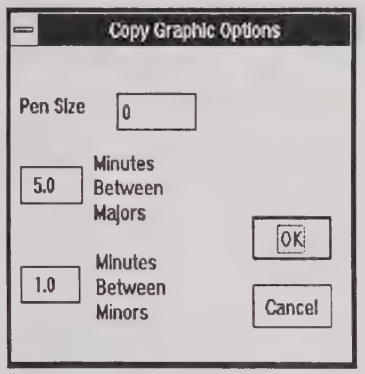
Copy

Copies the graphics or report text of the view in the editing window to the Windows Clipboard.



Copy Graphics...

The Copy Graphics Options dialog box determines the appearance of chromatograms transferred to word processing software, desktop publishing software, etc.



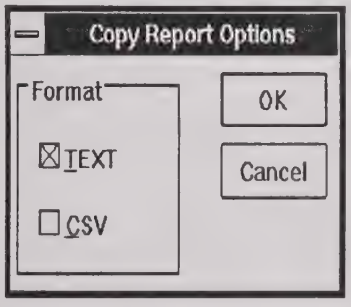
Pen Size controls the width of the chromatogram trace. The higher the number, the wider the trace and the more time required to print the chromatogram. If you use a laser printer, changing the value from 0 (the default) to 2 or 3 makes the line wide enough to be easily visible on slides or posters.

Minutes Between Majors sets the time interval between major time-scale ticks.

Minutes Between Minors sets the time interval between minor time-scale ticks.

Copy Report...

If you copy the report text to the Windows Clipboard, it can then be pasted into other software programs.



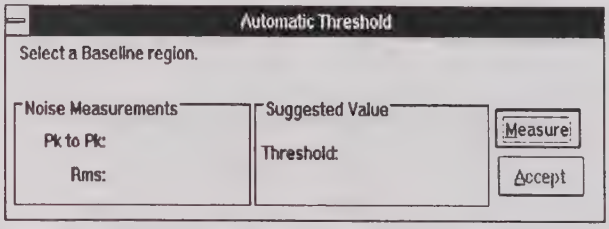
Select the **TEXT** format if you want to copy the report into a word processing program.

Select the **CSV** (Comma-Separated Variable) format if you want to copy the report into a spreadsheet or database program such as Excel or dBase.

Operations Menu

Auto Threshold

The automatic threshold feature evaluates a section of baseline, displays the average noise for the baseline, suggests a threshold that prevents the baseline from being interpreted as peaks, and, upon your approval, updates the current threshold.

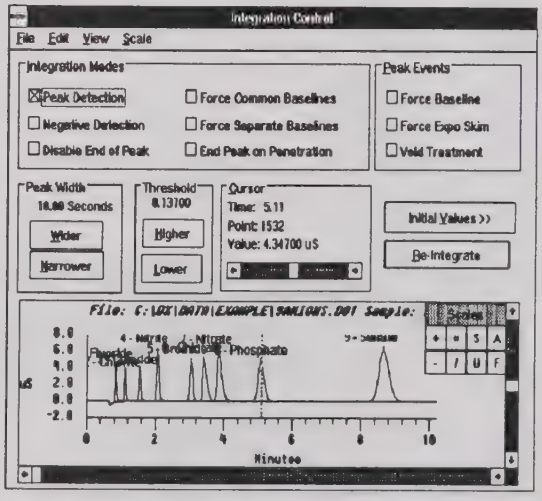


1. Check the chromatogram in the editing window for a peakless segment of baseline that you consider representative of the entire baseline. To select that region, place the mouse cursor at one end of the baseline segment, then press and hold the mouse left button while simultaneously dragging the cursor to the other end of the segment. Release the mouse button, and an enlarged view of the selected segment showing the average noise is displayed. (You may have to use Autoscale from the Scale menu or use the vertical scroll bar to bring the segment view onto the screen.)

2. Click on the Measure command button. A Least Squares Intercept Line representing an automatically determined optimum threshold will be displayed on the chromatogram. Two values (Peak-to-Peak and Root Mean Squared) and a suggested threshold value are displayed in the dialog box.

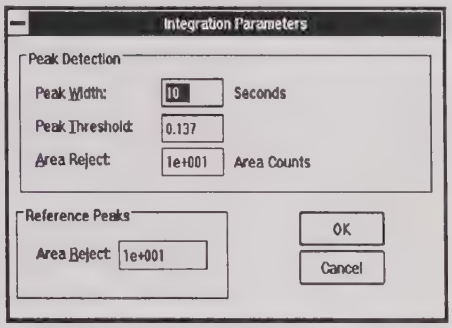
3. Click on the Accept command button to have the chromatogram integrated with the new threshold.

Adjust Integration...



The first step in optimizing the integration parameters for a data file is usually to select Data Events... from the Edit menu and delete all unwanted events from the file (see *Optimize/Edit Menu/Data Events...*).

The next step is to evaluate the initial integration parameters and make any changes required. To do so, click on the Initial Values >> command button. The Integration Parameters dialog box will appear superimposed over the Integration Control dialog box:



The options in this dialog box can be used to make basic changes that affect an entire chromatogram; for example, to change the Peak Width before using other integration features to fine-tune your data. The peak width should be approximately equal to the width (in seconds) at half-height of the narrowest peak in the chromatogram. The default Peak Width value (10) is appropriate for most liquid chromatography applications.

When you finish editing, click on OK to automatically integrate the data and return to the Integration Control dialog box.

Integration Modes

The controls listed in the Integration Modes section of the Integration Control dialog box represent six types of data events. All Integration Modes controls are implemented by moving the Time-Scale cursor to the point at which you want a control turned on (or off), clicking on the check box for that control, and then clicking on the Re-Integrate command button. This will modify peak integration throughout the rest of the run, unless you turn the control off (or on) before then. Each time you turn an Integration Modes control on or off, you are creating a **separate** data event.

NOTE: If you do not know how to set the Time-Scale cursor, refer to *Optimize/File Menu/Graphics Controls/Move the Time-Scale Cursor* for instructions.

Peak Detection detects peaks on the chromatogram. The control is normally on, but you may want to turn it off during a period of void volume column upset or the appearance of an apparent peak that you do not want reported.

Negative Detection detects negative peaks on the chromatogram. The control is normally off.

Disable End of Peak combines a group of peaks into one new peak. The new peak will be equivalent to the areas of the combined peaks.

Force Common Baselines forces a common baseline below a group of peaks so that areas of fused peaks are determined by drop-line integration.

Force Separate Baselines forces valley-to-valley baselines where drop-line integration was determined by the integration algorithm.

End Peak on Penetration If the integration algorithm causes the baseline to penetrate data on the plot, the End Peak on Penetration control brings the baseline to a logical position, relative to the data cluster that was penetrated.

Peak Events

Unlike the Integration Modes controls, which modify peaks throughout the rest of a run, the three controls listed in the Peak Events section of the Integration Control dialog box represent data events that modify only one specific peak.

Before using any of these controls, you need to understand the four types of peaks:

- **Type 1** has independent beginning and ending points on the baseline.
- **Type 2** shares at least one end with the end of another peak. The two peaks will have a drop line between them.
- **Type 3** is a well-defined peak, known as a mother peak, with an attached exponentially-skimmed peak.
- **Type 4** is an exponentially-skimmed peak attached to a mother peak.

Force Baseline forces a separate baseline between two Type 2 peaks where no baseline is evident. To activate the control, move the Time-Scale cursor onto the peak to the right of the area where you want to force a baseline, then click first on the Force Baseline check box and then on the Re-Integrate command button.

Force Exponential Skim If you separate a Type 3 (mother) peak from a Type 4 (child) peak by dropping a vertical line between them, the area allotted to the child peak is actually part of the tailing slope of the mother peak. The Force Expo Skim control extrapolates the exponential decay rate of the tailing slope of the mother peak, and then adjusts the areas of the mother and child peaks accordingly. Exponential skim is automatic if the height of the child peak is 20% or less of the height of the mother peak. Forced exponential skim is required in the following situations:

a) When the height of the child peak is 20% or more of the height of the mother peak.

b) When the height of the child peak is greater than three times the distance between the baseline and the valley between the mother and child peaks.

To activate the control, move the Time-Scale cursor to a point immediately before the Types 3 and 4 peaks you want to modify, then click first on the Force Expo Skim check box and then on the Re-Integrate command button.

Void Treatment When the upward slope of a peak starts at a negative data point, Void Treatment looks back along the baseline and starts the slope at the first non-negative data point encountered. This control is used chiefly to correct for water dips and void volume upsets.

To activate the control, move the Time-Scale cursor to a point immediately before the negative dip and then click first on the Void Treatment check box and then on the Re- Integrate command button.

Peak Width determines how many data points are combined into each data bunch. Increasing the peak width increases the number of data points per bunch, while decreasing the peak width lowers the number of data points per bunch.

Peak width requirements are directly interrelated with slope requirements, as explained in the section below on Threshold Selection. Narrow peaks require small data bunch sizes, to allow enough bunches across narrow peaks to permit peak detection. Wide peaks require large data bunches so that small changes in amplitude can be detected over long time intervals. If the peak width is narrow enough, the peak tick appears at the apex of the peak.

The Peak Width control is typically used to re-integrate a lengthy chromatogram in which narrow peaks increase to wide peaks as the run progresses. If this occurs, the chromatogram may be badly integrated, with the baselines of wider peaks ending too soon because the integration program had difficulty finding the true ends of the peaks. One solution is to increase the Peak Width setting before the first wide peak. This can also occur if the Threshold is set too high; see the section below entitled *Threshold Selection* for more information.

If a peak's tick mark is not at the peak apex, the Peak Width setting is probably too wide and you should select a narrower width. The best way to optimize integration is by on-screen experimentation.

Move the Time-Scale cursor to a point immediately before the area where you want to change the peak width, click the cursor on Wider or Narrower, as appropriate, and then click on the Re-Integrate command button.

Threshold Selection doubles or halves the current threshold.

The threshold is a value derived from the averaged slopes of data bunches sampled along a baseline sector. It is applied as a limiting slope to determine peaks. The integration algorithm measures the slope between every two data bunches in the entire chromatogram. It marks the potential start of a peak when two consecutive data bunches have a slope higher than the peak threshold value; if a third data bunch exceeds the threshold value, the algorithm marks the start of a peak.

The algorithm continues to view data and look for the top of the peak. It determines the top of a peak when the slope of the sampled data drops below the positive threshold value. The end of a slope is determined when data bunches reach and maintain the threshold value.

The threshold value normally changes very little from the value provided by the Auto Threshold control, but you may occasionally find it necessary to make minor changes to balance modifications to peak width or to compensate for increased noise levels.

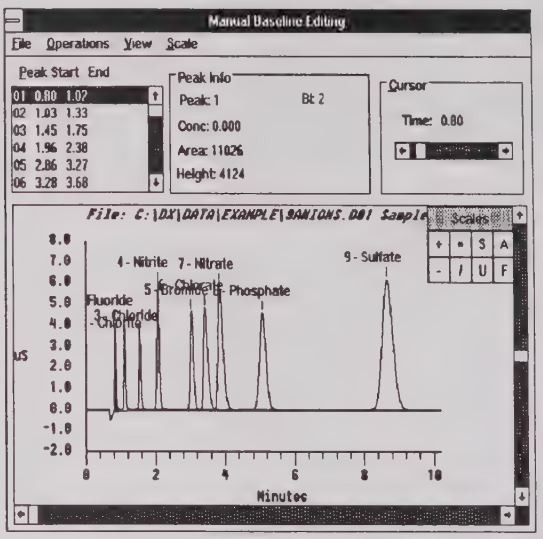
To do so, move the Time-Scale cursor to a point immediately before the area where you want to change the threshold value, select the new value by clicking the mouse left button on Higher or Lower, as appropriate, and then click on the Re-Integrate command button.

Cursor Move the Time-Scale cursor by positioning the mouse cursor on either the right or left arrow of the scroll bar and then pressing the mouse left button. The number of data points and value for the selected time will automatically be displayed.

Set Baselines Manually...

Any changes made with the Optimize graphic controls discussed previously can be saved to the Method and applied to other chromatograms. Unlike those controls, Manual Baseline Editing adjusts the baseline **only** for the file you are working on.

The File, Operations, View, and Scale menus are listed on the menu bar of the Manual Baseline Editing dialog box. Only the Operations menu has new commands specific to this dialog box; these new commands are explained below. For more information about the function and use of commands on the other menus, refer to the appropriate sections in this chapter.



Peak Selection lists the number, starting time, and ending time for each peak in the file. Use one of these three methods to select a peak whose baseline you want to modify: click the mouse left button on the peak number, select the peak on the chromatogram with the mouse right button, or click the mouse right button on the peak in the editing screen. An enlarged view of the selected peak and baseline is displayed on the editing screen. Double-clicking enlarges the view of the peak and baseline.

Peak Info Whenever you select a peak for modification, the current peak number, baseline type, concentration, area, and height are immediately displayed in this section.

Cursor Move the Time-Scale cursor by positioning the mouse cursor either on the right or left arrow of the scroll bar and pressing the mouse left button. Scroll speed can be increased by a factor of ten by pressing the <Alt> key while operating the scroll bar.

Operations Menu

This section describes only the new Operations menu commands available while working in the Manual Baseline Editing dialog box. For information about other Operations menu commands, refer to *Optimize/Operations Menu*.

NOTE: Turning on the Start/Stop Markers option listed on the View menu makes it easier to locate the beginning and end of a peak.

- **To change the start time of a peak,** move the mouse cursor along the chromato-gram toward the start of the selected peak until the cursor changes to a double-ended arrow; this arrow indicates the start of the peak. Press the mouse left button, drag the cursor to the point on the time-scale where you want the peak to start, and release the mouse button.
- **To change the end time of a peak,** drag the double-ended arrow cursor from the end of the peak to the new position where you want the peak to end.

Split Peak adds a new peak by splitting an existing peak into two. Position the Time-Scale cursor at the point you want the peak split and select the Split Peak command.

Delete Peak deletes a selected peak from the Peak Table and from integration results.

Merge Peak Right combines the areas of the selected peak and the peak adjoining it to its right on the time-scale. The combined right peak loses its separate identity as a peak.

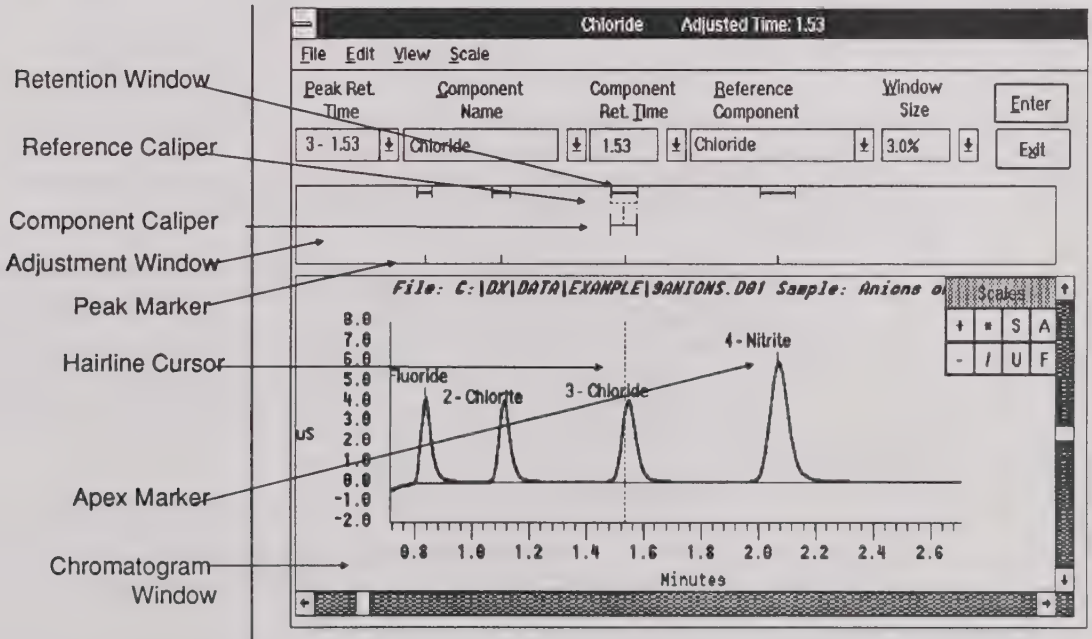
Merge Peak Left combines the areas of the selected peak and the peak adjoining it to its left on the time-scale. The combined left peak loses its separate identity as a peak.

Move Peak Start Position the Time-Scale cursor at the new point where you want the peak to start and then select the Move Peak Start command. You can also move the beginning of a peak by using the Peak Start/End cursor described below.

Move Peak End Position the Time-Scale cursor at the new point where you want the peak to end and select the Move Peak End command. You can also move the end of a peak by using the Peak Start/End cursor described below.

Re-Detect Peaks deletes the results of all baseline changes made to a chromatogram during editing and returns the chromatogram to its previous configuration.

Name Peaks...



Use this dialog box to do the following:

- Create a new component table by entering names for the peaks of a chromatogram
- Add new components to an existing component table
- Match unidentified or misidentified peaks to the correct components
- Adjust retention time windows and component references graphically to ensure proper peak identification

Selecting a Peak

Position the cursor over the peak and press the mouse **right** button. A hairline cursor appears over the peak, and information about the peak appears in the boxes at the top of the dialog box. If the peak is identified as a component, the Adjustment Window displays the Component Caliper, the Reference Caliper (if there is a reference component), and the Retention Window Marker.

Selecting a Component

Use the arrow to the right of the Component Name drop-down list box to display available component names, then click the cursor on the desired name to select that component. You can also select a component by clicking the cursor on the Retention Window Marker above the desired component.

Naming Unidentified Peaks

After selecting the peak of interest, open the drop-down list box under Component Name to see whether the peak component is already listed.

If it is listed, select it to display the current component information. Update the component time, using one of these methods:

- Select the new time from the Component Time drop-down list
- Use the mouse to move the Component Caliper over the correct peak
- Type the new component time in the Peak Ret. Time edit box

If the component name is not listed, type the name in the Component Name box and press <Tab> to enter the peak time as the Component Time.

Edit the Reference Component and Window Size, if desired, then press <Enter>. If the window size is a percentage, remember to also enter a percent sign; a value without a percent sign will be interpreted as an absolute window (expressed in minutes). The new component assignment is entered in the component table and should appear in the scalable plot. If the new component assignment does not appear, the reference component has probably shifted too much. See *Renaming Misidentified Peaks*, below, for a corrective procedure.

Renaming Misidentified Peaks

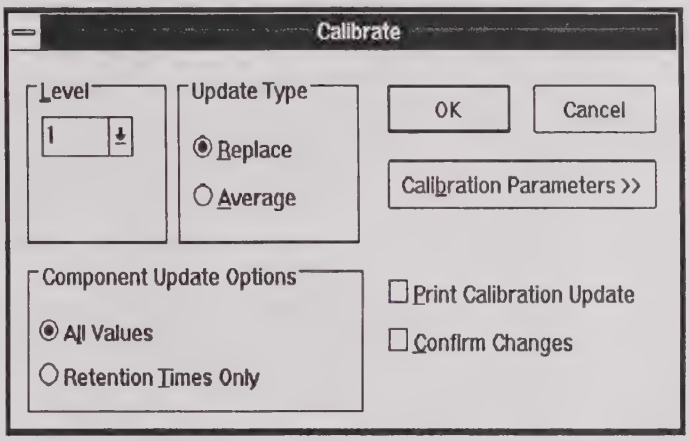
Peaks can be misidentified because of a problem with the reference component, an excessively wide Retention Window Marker, or a mismatch between the expected and actual retention times.

To rename a misidentified peak, first select the peak of interest. The Component Caliper will appear above the peak. If a reference component is shown, make sure that the Reference Caliper is approximately centered over the reference peak. If it is not, the shift may be causing too large an adjustment in other component windows. To correct this, select the reference peak and move the Component Caliper until the Retention Window Marker is centered over the Component Caliper.

If any Retention Window Markers overlap each other, reduce their widths by selecting them with the mouse, selecting or typing in a narrower Window Size, and pressing <Enter>. Continue until no overlaps are present.

Calibrate

This option is used to calibrate the Method with the current data file. You can update the height, area, and retention time values for each peak, or just the retention times. Calibrate is normally used after you have completed all other adjustments to the Method.



Level displays a drop-down list box in which you select the calibration level (1 to 10).

Update Type

Replace updates the Method by replacing the values currently stored in it with the values determined by the current integration of the current data file.

Average updates the Method with values obtained by averaging the values currently in it with values in your data file.

Component Update Options

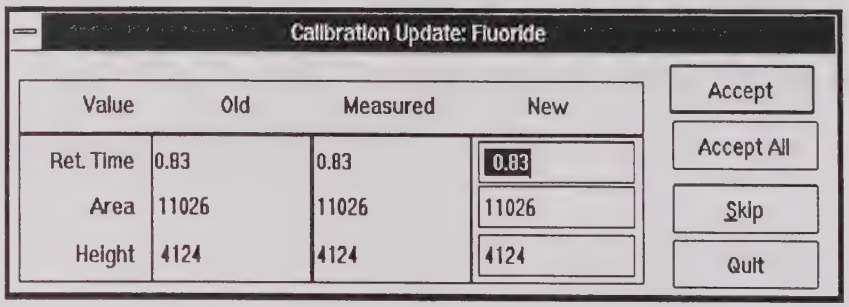
All Values updates all values in the Method.

Retention Times Only updates the retention times in the Method but leaves the calibration unchanged.

Calibration Parameters >> displays the same Calibration dialog box that is opened when you select Calibration... from the Edit menu or in the Data Processing section of the Method Editor program.

Print Calibration Update sends current data to the printer.

Confirm Changes Click on Confirm Changes, and then on OK, to generate a dialog box prompting you to approve values for each component as you calibrate:



Accept means you accept the new values for a component.

Accept All means you accept new values for **all** components, including those not displayed.

Skip lets you skip this component without accepting a new value.

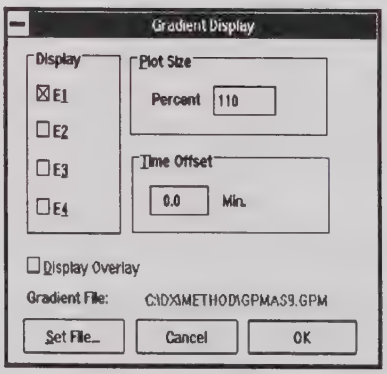
Quit abandons the procedure, although changes already accepted remain in effect.

View Menu

Reference Plot, Grid, Baselines, Start/Stop Markers, Peak Fills

These commands control the graphics features of the corresponding names. To turn a feature on or off, click the mouse cursor on the command.

Gradient Display...



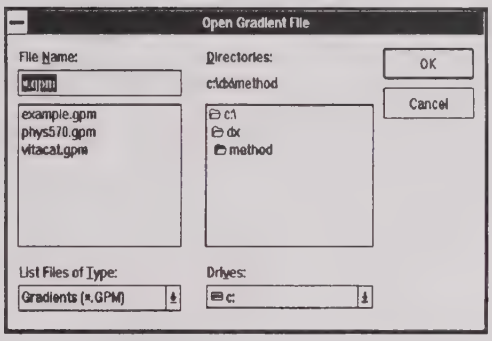
Display selects an eluent trace for plotting. Click the mouse cursor on the appropriate check box(es).

Plot Size sets the size of the gradient plot offset. To change the size, erase the displayed value by using the <Backspace> or <Delete> key, and then type in a new value.

Time Offset specifies a time offset that adjusts the gradient plot to compensate for the time it takes the gradient mixture to reach the column after it is generated by the pump. A 2-minute delay is typical. To change the time offset, erase the displayed value by using the <Backspace> or <Delete> key, and then type in a new value.

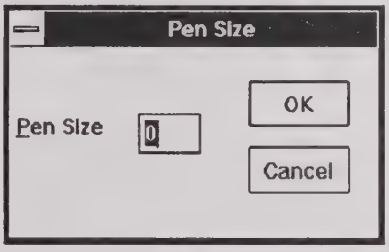
Display Overlay shows or hides the gradient display. When this option is selected, the current gradient is superimposed over the chromatogram in the editing window. The default gradient is the one used by the Method stored with the data file. Use Set File... to select a different gradient file.

Set File... opens the Open Gradient File dialog box, from which you can select a gradient file for use with the currently displayed chromatograph.



When you finish making selections in the Gradient Display dialog box, click the mouse cursor on OK to have your changes accepted.

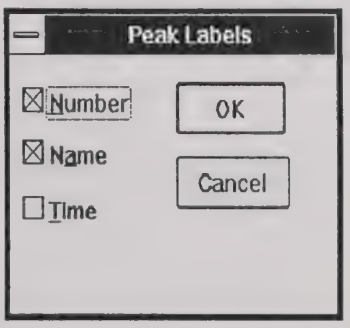
Pen Size



Pen Size controls the width of the chromatogram trace: the higher the number, the wider the trace and the longer the time required to print the chromatogram. If you are using a laser printer to prepare overhead projection slides, change from 0 (the default value) to 2 or 3. To change the size, delete the displayed value by using the <Backspace> or <Delete> key, then type in a new value.

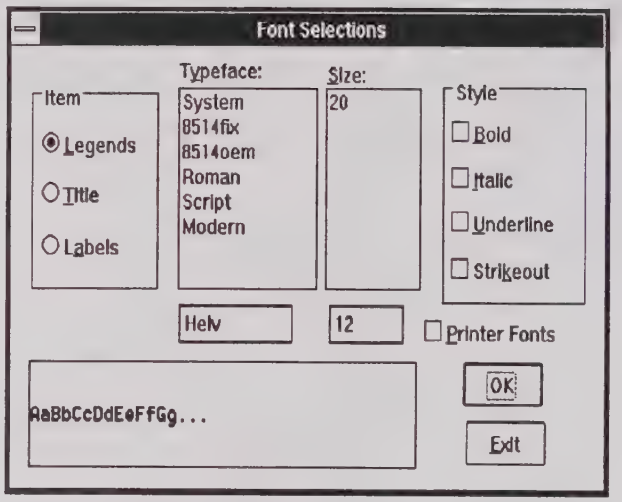
Peak Labels...

To have the number, name, and/or retention time of a peak included in the peak label, click on the corresponding check box here.



Fonts...

Use the Font Selections dialog box to select the font styles and sizes for the printed chromatogram.



Click on the Printer Fonts check box to list only those typefaces and sizes that are supported by the printer installed in Windows Control Panel, rather than the display fonts that normally are listed. If you try to print a chromatogram using a display font not supported by your printer, the printer usually uses a substitute (and often undesirable) font.

In the Item section, **Legends** are the scale numbers along the X and Y axes, **Title** is the filename across the top of the chromatogram screen, and **Labels** are the numbers and names that identify peaks.

Select an Item, and then select its Typeface, Size, and Style. The result of your selections will be displayed in the preview window in the lower left corner of the dialog box. If you like the appearance of these selections, click on OK to apply them. Repeat this process for any other Items.

When you are finished, click on the Exit command button.

Scale Menu

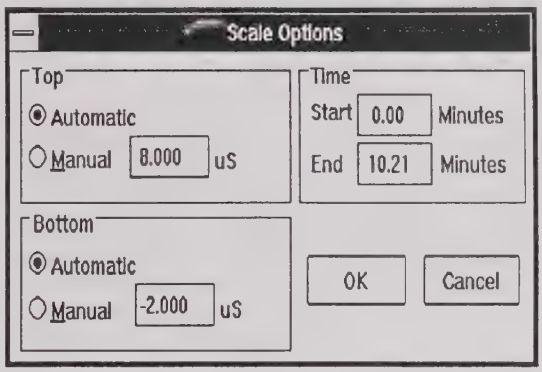
The Scale menu commands are used to select viewing options. There are three ways to select a command:

1. Click on the name under the menu.
2. Key the character, or <Control> (^) plus the letter listed after the control name on the menu.
3. Click the cursor on the character representing the control in the Scales Toolbox.

Undo

Undo causes the editing screen to revert from its present scale to the previously set scale.

Scales...



Sets a chromatogram's scales directly.

Top and Bottom

When you select **Automatic**, the software looks for the maximum value of the chromatogram and uses that value as the top of the chromatogram.

Select **Manual** and enter the desired value when you want to look at a particular segment of the baseline.

Time Enter the Start and End times (in minutes) for the segment of baseline you are interested in. You can choose a time scale greater than that of the chromatogram.

Full View

Displays the entire chromatogram in the editing window.

AutoScale

Automatically sets Scale to Highest Peak and Scale Bottom to Lowest Value. This maximizes peak sizes and contrast.

Zoom

Zoom In Vertically (+) increases the distance between the Response Axis scale ticks, thereby showing a narrower section of the chromatogram at greater vertical magnification.

Zoom Out Vertically (-) decreases the distance between the Response Axis scale ticks, thereby showing a broader section of the chromatogram at less vertical magnification.

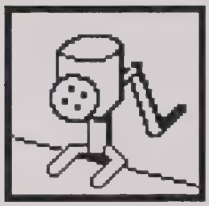
Zoom In Horizontally (*) increases the distance between the Time Axis scale ticks, thereby showing a narrower section of the chromatogram at greater horizontal magnification.

Zoom Out Horizontally (/) decreases the distance between the Time Axis scale ticks, thereby showing a broader section of the chromatogram at less horizontal magnification.

Scale Tools

Shows or hides the toolbox display in the upper right corner of the editing window.

What Is Batch?



The Batch program is used to reprocess groups of data files and to subtract a baseline from a group of data files. Both procedures require a Schedule that lists the data files and Methods to be used. This Schedule can be the same one used by the Run program when the data was acquired during automated sample analysis (unless you have changed data filenames since then) or, if you prefer, you can create a new Schedule, from within Batch, specifically for the reprocessing operation. After reprocessing, Batch can store the reprocessed data, print reports and chromatograms, save the result reports for each file, and update the Method(s).

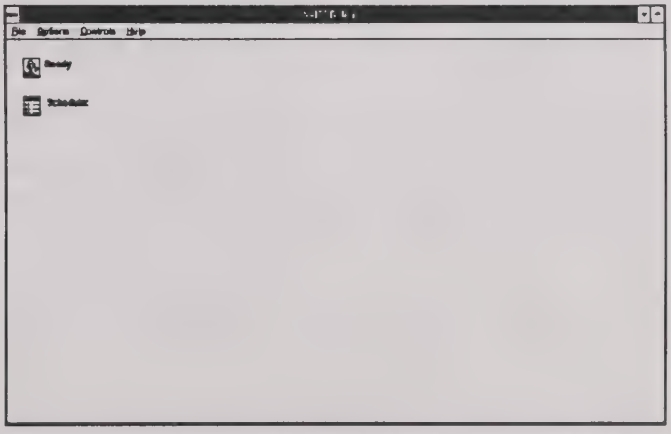
Batch can also print out a statistical summary for the reprocessed data files which will include the total number of samples in which each component in the Method Component Table was found, as well as the mean, variance, standard deviation, and relative standard deviation of the concentrations, peak areas, and peak heights.

Finally, Batch can organize the information in each data file into spreadsheet format for manipulation in spreadsheet editors that can read files in standard ASCII format (comma-separated ASCII variables).

The Batch program creates and uses the following files:

Files Used	Files Created	Comment
*.ME?		Methods
*.SCH	*.SCH	Schedules
*.D??	*.D??	Raw data files
*.DAT	*.DAT	DataMath raw data files
*.BCH	*.BCH	Batch Settings File
	*.CSV	File exported to Microsoft Excel
	*.PRN	File exported to Lotus 1-2-3

Starting the Batch Program



To start Batch, double-click on the Batch icon in the AI-450 MainMenu or on BATCH.EXE in the directory that holds your AI-450 files. The commands, dialog boxes, and windows associated with each Batch menu are explained on the following pages.

BATCH.EXE Command Summary

File Menu
- New
- Browse Data Files...
- Open Batch Settings File...
- Save Batch Settings File...
- Select Existing Schedule File...
  - Batch Reprocessing Options
- Build Batch Schedule...
  - Data File Selector
    - File Menu
      - Save Selected Files...
      - Delete Selected Files...
- View Schedule...
- Printer Setup...
  - Control Panel

Options Menu
- Processing Options...
  - Input Options
  - Print Options
  - Update Options
  - Math Processing
- Export Options...
  - Create Export File During Batch Job
  - Export Filename
  - File Type
  - Report
  - Fields >>
    - Header Fields
    - Data Fields

Controls Menu
- Start

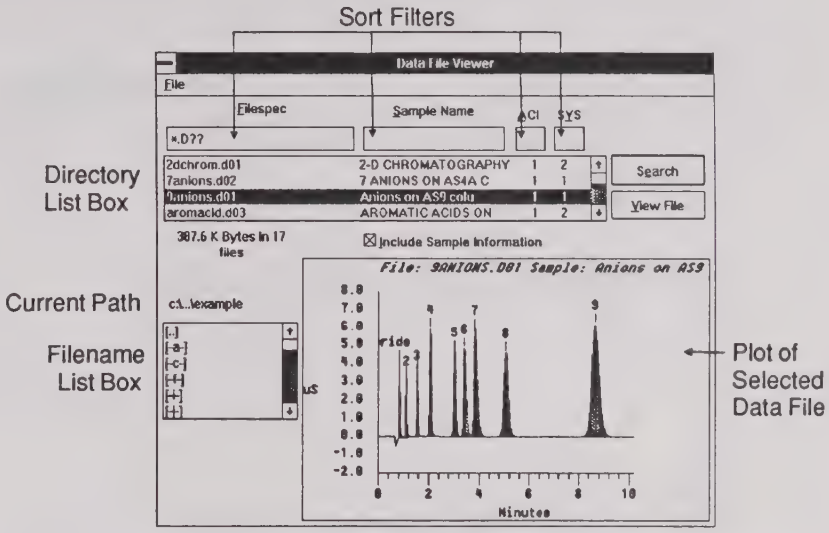
File Menu

New

New clears any option you selected while working on a file with Batch and resets the program to the conditions previously in effect.

Browse Data Files...

This option lets you quickly view the chromatograms of data files. You can select and view samples as you scroll through the filename list box.



Select Directory Click on the desired disk drive and directory in the directory list box.

Select File Select samples by clicking on the filenames as you scroll through the filename list box. To restrict your search to just those samples in which you are interested, enter sort filters.

Sort Filters simplify your search for specific samples by filtering out all files which do not match the criteria entered in the Filespec, Sample Name, ACI, and SYS fields.

Filespec (Filename) Type a filename of one to eight characters (no punctuation or spaces) and the extension .D??, where ?? is a number from 01 to 99 and corresponds to the inject number in the Schedule. If you leave the filename list box blank, the names of all raw data files in the current directory are displayed.

Sample Name Type a sample name of one to eighteen characters (no punctuation or spaces).

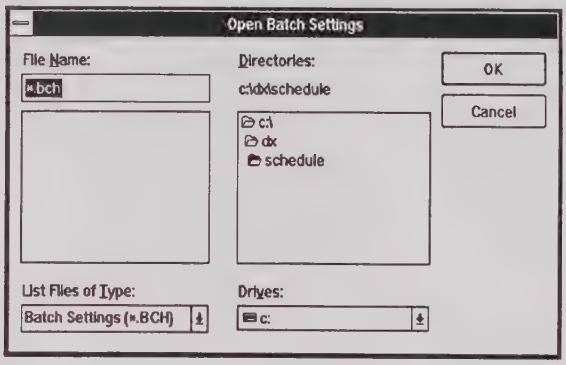
ACI/SYS Depending on which model data system you are using, select an Advanced Computer Interface (ACI), 1 through 6, and system 1 or 2. If you leave these boxes blank, selected files for all interfaces and systems are displayed.

Wild Cards are two special characters, ? and *, that can be used when entering sort filters. The ? indicates that any character can occupy that position. The * indicates that any character can occupy that position and all the remaining positions in the name, extension, or number field being entered. Wild cards cannot be used with sample names.

Include Sample Information displays or hides the Sample Name, ACI, and SYS information. File updating is much faster if sample information is not included.

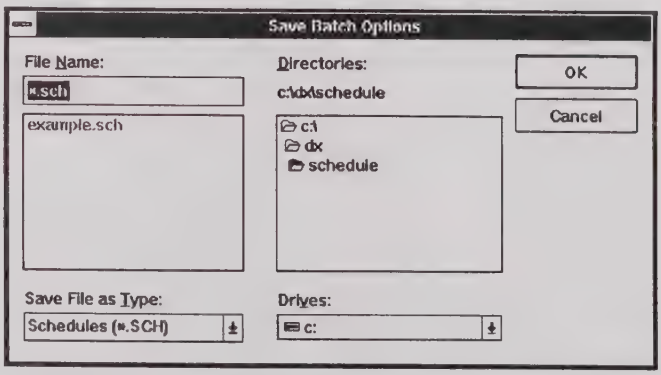
Open Batch Settings File...

A batch settings file is a convenient tool for saving the specifications (Schedule name, reprocessing options, export options, etc.) for a particular batch reprocessing job. It is not necessary to use batch settings files, but if you expect to repeatedly reprocess the same types of data, they can simplify setting up the job.



Save Batch Settings File...

Once you select all the specifications for a reprocessing job, you can save them in a separate file for use with other jobs. Select Save Batch Settings File... to open this dialog box:



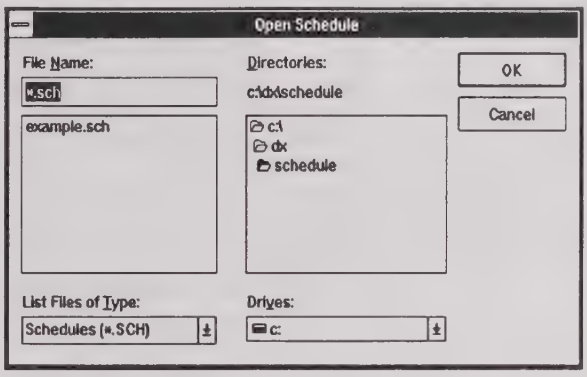
Type in a filename of one to eight characters (no punctuation or spaces). The extension ".BCH" is added automatically to identify the file as a batch settings file. Unless otherwise specified, the file is stored in the \SCHEDULE subdirectory in the directory that holds your AI-450 files. You may change the pathname, if desired.

Select Existing Schedule File...

After data acquisition, you may want to modify your Method in order to optimize the analytical results and then use the modified Method to batch reprocess all of the data. Batch reprocessing is always carried out under the control of a Schedule. Unless the data filenames have been changed, this is often the same Schedule used for data acquisition.

The Select Existing Schedule File... command displays the Open Schedule dialog box. When you specify an existing Schedule to use during batch reprocessing, the Batch Reprocessing Options dialog box appears (see *Batch/Options Menu/Processing Options...*).

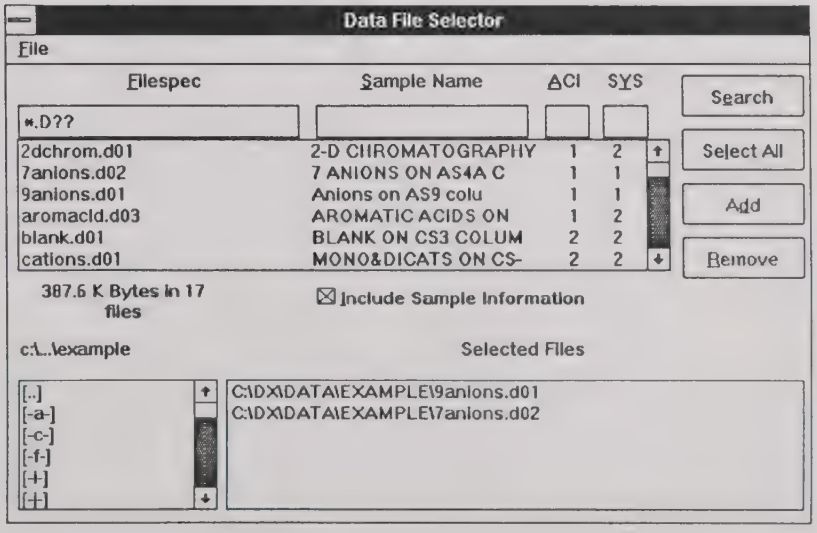
NOTE: If you have a dual-detector system, build a separate batch Schedule to reprocess data from Detector 2.



Build Batch Schedule...

Use this command to create a new Schedule specifically for raw data files that require reprocessing. Use the list boxes in the Data File Selector dialog box (see *Batch/Edit Menu/Browse Data Files...*) to select the directory and raw data files.

NOTE: If you have a dual-detector system, build a separate batch Schedule to reprocess the data from Detector 2.



The File menu of the Data File Selector dialog box lists two options:

Save Selected Files As... saves the list of files that you have selected to reprocess a Schedule. The default filename is BATCH.SCH. Unless you specify otherwise, this file will be added to the \DX\SCHEDULE directory. The files will be reprocessed in the order in which they appear in the Selected Files list box.

Delete Selected Files... deletes (from disk) files listed in the Selected Files list box.

View Schedule...

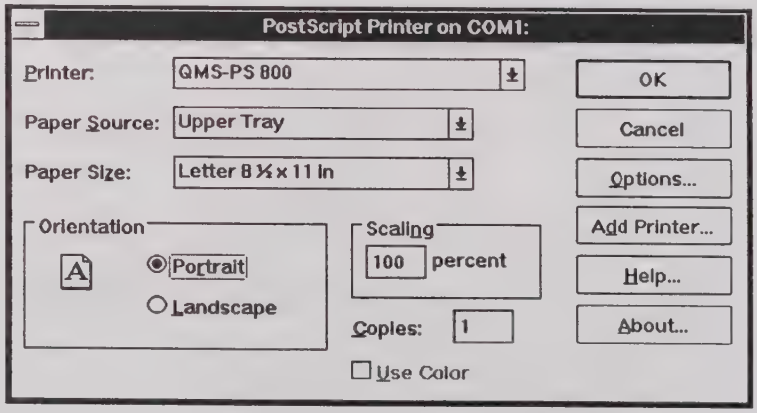
Opens the Schedule Editor program and pre-loads BATCH.SCH, the default Schedule, into the worksheet.

Edit BATCH.SCH as you would any other Schedule. The values in the Injection Volume, Dilution Factor, and Internal Standard cells affect the calculated concentration values exactly as they do when the data is first processed by Run. To edit a different batch Schedule, use the Open command from the File menu.

NOTE: Sample names entered in the Schedule will be saved in the data files if you select the Re-Store Data After Reprocess option (see *Batch/Options Menu/Processing Options*).

Printer Setup...

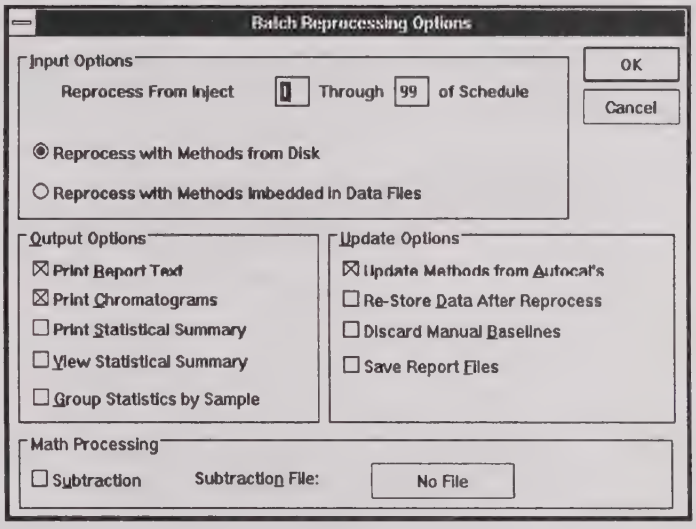
Opens the Printer Setup menu of the Windows Control Panel (CONTROL.EXE). The menu depends on which printer was installed, so yours may differ from the example here. Refer to your Windows documentation for instructions for using the Control Panel.



Options Menu

Processing Options...

Processing options let you specify which files you want reprocessed, the type and content of any printed reports, and what is done with the raw data after it is reprocessed. When you choose the Select an Existing Schedule File... command from the File menu, the Batch Reprocessing Options dialog box appears automatically:



Input Options

Reprocess From Inject specifies the first and last injections to be reprocessed. Each line in the Schedule worksheet represents one injection (a Schedule can include a maximum of 99 injections). Enter the desired line numbers in these edit boxes.

If you select **Reprocess with Methods from Disk**, the most current Methods from disk will be used. If you select **Reprocess with Methods Embedded in Data Files**, Method parameters embedded in data files will be used.

Output Options determine the contents of printed reports.

Print Report Text prints a result report for each data file after the raw data is reprocessed.

Print Chromatogram prints a chromatogram showing retention times and baselines for the reprocessed data. (Note that the Batch program runs significantly faster when chromatograms are not printed.)

Print Statistical Summary prints a statistical summary for each of the components in the Method calibration table (see the example below). These statistics include the total number of samples in which the component was found, as well as the mean, variance, standard deviation, and relative standard deviation of the peak areas, peak heights, and concentrations.

```
                    Batch Reprocess Statistical Summary
Schedule File: C:\DX\DATA\PAX500.SCH
Cycle 01 to 26
Data Processed on  Mon Jul 22  13:49:22 1991
```

Component: Fluoride
N = 26

	Amount	Area	Height	Ret. Time
Averages	1.988	2.265e+007	4113151	2.00
Variance	0.003	3.348e+011	11483712704	0.00
Std Deviation	0.051	5.786e+005	107162	0.00
% Rel.Std.Dev.	2.55%	2.55%	2.61%	0.00%

Component: Chloride
N = 26

	Amount	Area	Height	Ret. Time
Averages	3.008	2.954e+007	4061798	2.62
Variance	0.006	5.323e+011	6227152374	0.00
Std Deviation	0.074	7.296e+005	78912	0.00
% Rel.Std.Dev.	2.47%	2.47%	1.94%	0.12%

Component: Sulfate
N = 26

	Amount	Area	Height	Ret. Time
Averages	9.945	6.626e+007	7845029	3.63
Variance	0.020	8.662e+011	41999302849	0.03
Std Deviation	0.140	9.307e+005	204937	0.02
% Rel.Std.Dev.	1.40%	1.40%	2.61%	0.59%

View Statistical Summary displays the statistical summary in a viewing box when the batch job is completed.

Group Statistics by Sample generates separate statistical summaries (like the one above) for each group of sample replicates, instead of one summary for the whole Schedule.

Update Options determine how disk files are updated.

Update Methods from Autocal's updates the component table of each Method(s) used to reprocess the data whenever a data file with the sample name AUTOCAL is processed. This update provides revised peak heights and peak areas for each component.

Re-Store Data After Reprocess stores reprocessed data files on disk. The original data points and the current Method parameters are stored under the same filename as the original data. **Note that the original file is replaced.**

Discard Manual Baselines re-integrates data files with baselines that were manually modified by Optimize. Selecting this option re-integrates the chromatograms with automatically detected peaks.

Save Report Files saves to disk a result report for each reprocessed raw data file. The result report includes peak heights and areas, based on current baselines. The result report file will have the same filename as the raw data file but with the extension ".R??", where ?? is a number from 01 to 99.

WARNING! If a result report file with the same name is already stored in the specified directory, it will be overwritten.

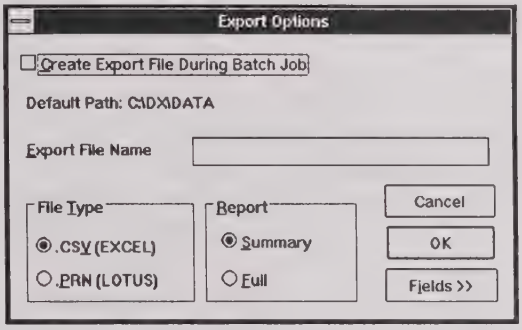
Math Processing

Subtraction subtracts a selected data file from all of the data files to be batch processed. To select a file to be the subtrahend, click the mouse cursor on the No File button to open a list box, then select the file by highlighting its name and clicking the mouse cursor on the Open command button.

WARNING! If you select both Subtraction and Re-Store Data After Reprocess (from Update Options), the data file written to disk will be the result of the subtraction: YOUR DATA FILE WILL BE MODIFIED.

Export Options...

Summary reports can be generated for export to Microsoft Excel, Lotus 1-2-3, or other programs that can read ASCII character files in a comma-separated variable format. The summary reports can be easily manipulated to produce a variety of reports and graphs.



Create Export File During Batch Job specifies whether you want to create an export file.

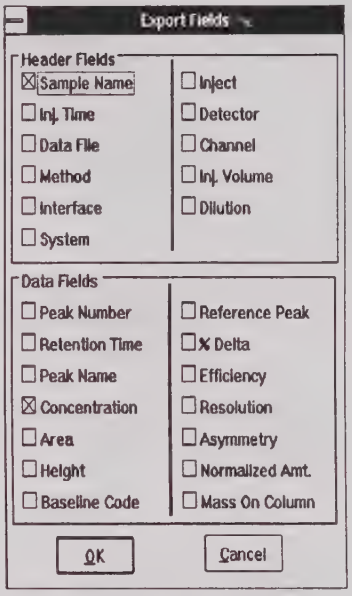
Export Filename Enter a pathname and a filename of one to eight characters (no punctuation or spaces) for the export file to be created. If you do not include a pathname, the file will be stored in the \DX\DATA directory.

File Type selects the format of the export file. Options are the ".CSV" format used by Microsoft Excel or the ".PRN" format used by Lotus 1-2-3. The specified type is added as the extension for the export filename.

Report determines whether the exported file is a full report or just a summary. A full report exports information in the same format used by the result report generated after data processing. Summary reports are typically more useful because they write one data record per raw data file into a flat-file database.

Fields>>, which is active only when Report Summary is selected, opens the Export Fields dialog box.

Use the Export Fields dialog box to specify which information from each result report is included in the export file.



The items in Header Fields are from the Report Header and are used as a header for each data record. The items in the Data Fields specify which information about each named sample component is included in the export file.

Here is the exported text file for the fields selected above.

```
"Sample Name","fluoride Conc","chloride Conc","nitrite Conc","bromide
Conc","nitrate Conc","phosphate Conc","sulfate Conc",
"Anions on AS4A column",15.1847,3.89815,0,0,0,0,0,
"Anions on AS9 column",0,0,0,0,0,0,0,0,0,
```

Importing Files Into Microsoft Excel

1. Start the Excel program.
2. Select the Open... command from the Excel File menu.
3. Enter *.CSV as the file specification.

   NOTE: If you have an international version of Excel, substitute the local-language equivalent extension for a comma-separated variable file.
4. Select the directory in which you stored the export file from the Batch program, then choose or type in the appropriate filename.
5. Click on the Open button. Excel will load the export data file into a spreadsheet.

Importing Files Into Lotus 1-2-3

1. Start Lotus 1-2-3.
2. Choose the /File Import Numbers command from the main Lotus menu.
3. Select the directory in which you stored the export file from the Batch program, then choose (or type in) the appropriate filename.
4. Lotus will read the export data file into a worksheet, starting at the current cell location.

   NOTE: Lotus has a maximum width limit of 132 characters. If the export file cannot be loaded, try importing a file with fewer data fields.

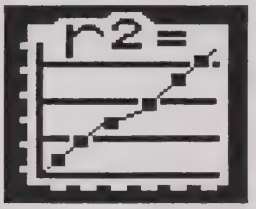
Controls Menu

Start

Starts the batch reprocessing job.

Batch verifies that all of the required files exist and then begins reprocessing the raw data files, as specified in the Schedule, and generating all of the requested reports.

If a required Method file is not on disk, Batch will ask if you want it extracted from a data file. If you answer yes, and if the original directory is still present, the Method is saved on disk in that directory.



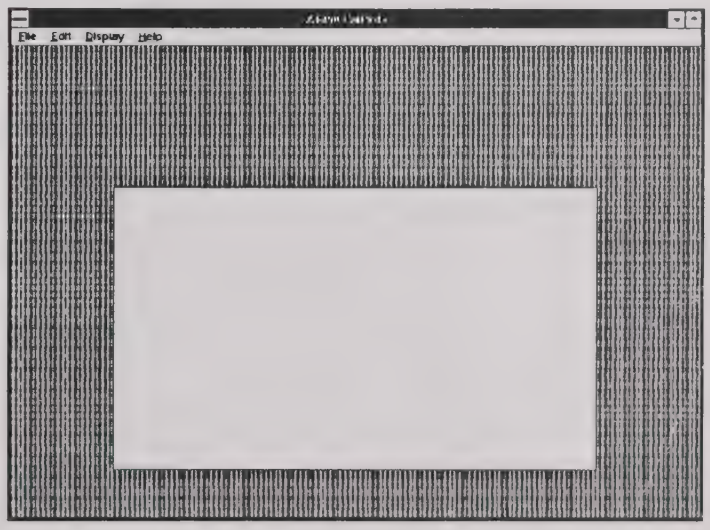
The CalPlot program plots the response vs. amount calibration curve for each component of a Method that uses multilevel calibration. The calibration is typically performed by the Run program's Automatic Calibration feature, using AUTOCAL calibration runs, but may instead be performed manually with the Calibrate feature in Optimize. There are four possible fit types, plus a "force zero" linear fit which intersects the origin (see *Method Editor/Calibration*). You can print a hard copy of the calibration plot for each component of the calibration standard.

A box above each calibration plot lists the component name, the calibration fit type, and, when applicable, the mathematical equation used to calculate the amount and response. A plot of the residuals (i.e., the differences between the actual calibration points and the calculated curve) and the sum of the residuals can also be viewed. For a detailed discussion of the equations used for determining calibration curves, refer to Appendix C.

The calibration curve and any additional information in the box above the plot are used primarily to verify the linearity of a Method over the concentration range for which it was developed. Regardless of the fit type selected for the Method, r^2 (the coefficient of determination) provides a measure of the curve fit. If $r^2 = 1$, the correlation is a perfect fit.

Starting the CalPlot Program

To start the CalPlot program, double-click on the CalPlot icon in the AI-450 MainMenu or on CALPLOT.EXE in the directory that holds your AI-450 programs. The CalPlot main window will appear, with four pull-down menus. The window is initially blank. When you open a Method, the response vs. amount calibration plot of the first component in the Component Table is displayed and additional information about the plot appears in a box above the plot. Use the scroll bar at the bottom of the window to select other components in the table.



CALPLOT.EXE Command Summary

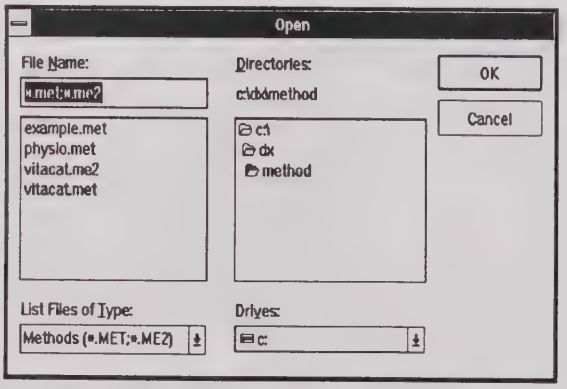
File Menu
- Open Method...
- Save Method As...
- Print...

Edit Menu
- Component Table...
- Calibration...
- Calculator...
- Copy...

Display Menu
- Components
- Residuals

File Menu

Open Method...

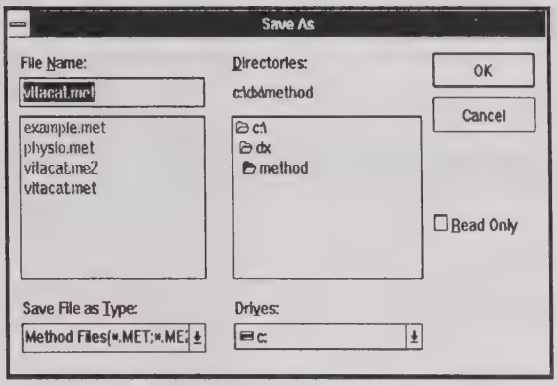


Method files are usually stored in the C:\DX\METHOD directory under filenames that include the extension ".ME?", where ? is either "T" for a Detector 1 Method or "2" for a Detector 2 Method.

Use the Directories and Drives list boxes to open the Method file. Double-click on the desired filename or type in the name (e.g., METHOD.MET), then click on the OK button or press [Enter].

Save Method As...

This command saves changes made to the Component Table and/or Calibration Parameters, either under the original Method name or under a new name.

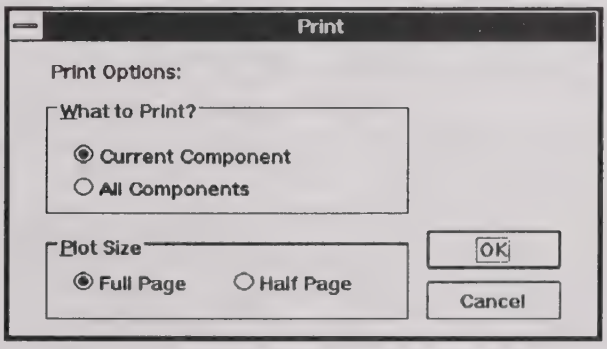


Enter a filename of no more than eight characters (letters and/or numbers, no punctuation or spaces) to identify the Method. The Method Editor program automatically adds the subdirectory \METHOD and the extension .MET to the filename. If the Method is for the second detector, Method Editor automatically adds the extension ".ME2" to the filename, but you may change the pathname, if desired. The filename will be stored in the directory that holds your AI-450 files.

NOTE: Refer to Appendix E for more information about the use of directories and pathnames for organizing your files on disk.

Print...

Prints the calibration data and the currently displayed plot(s), response, amount, and/or residuals for specified components in the Method Component Table.



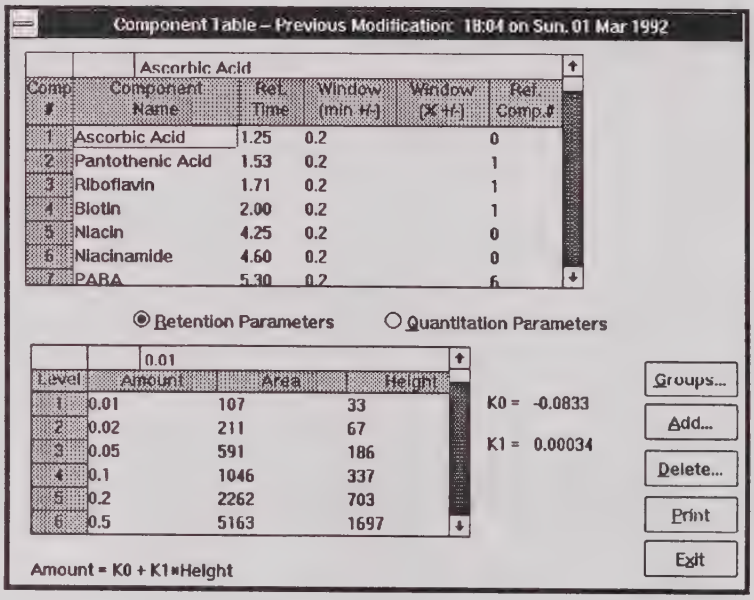
What to Print? determines whether the printout consists of just the component currently displayed or whether it includes the plots of all components in the Component Table. If you want to print residuals, select the Residuals option from the Display menu, described later in this chapter.

Plot Size selects a full-page or half-page size for the printed plot.

Edit Menu

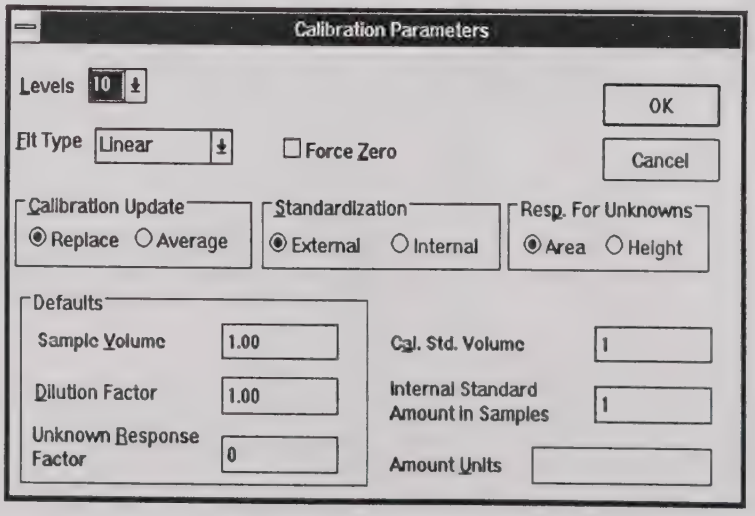
Component Table...

Opens the Component Table dialog box from the Method. You can view, edit, add, and delete information to change the calibration. For detailed information about the Component Table, see *Method Editor/Components*.


Component Table – Previous Modification: 18:04 on Sun. 01 Mar 1992
Ascorbic Acid
Comp # | Component Name | Rel. Time | Window (min +/-) | Window (% +/-) | Rel. Comp.#
1 Ascorbic Acid 1.25 0.2 0
2 Pantothenic Acid 1.53 0.2 1
3 Riboflavin 1.71 0.2 1
4 Biotin 2.00 0.2 1
5 Niacin 4.25 0.2 0
6 Niacinamide 4.60 0.2 0
7 PABA 5.30 0.2 6
Retention Parameters
Quantitation Parameters
0.01
Level | Amount | Area | Height
1 0.01 107 33
2 0.02 211 67
3 0.05 591 186
4 0.1 1046 337
5 0.2 2262 703
6 0.5 5163 1697
K0 = -0.0833
K1 = 0.00034
Groups...
Add...
Delete...
Print
Exit
Amount = K0 + K1*Height

Calibration...

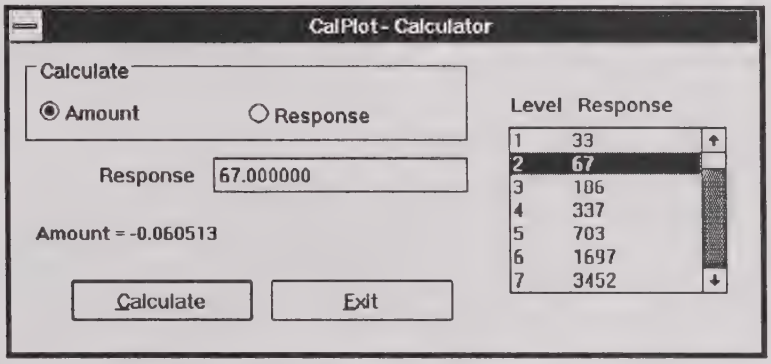
Opens the Calibration Parameters dialog box for the Method currently in computer memory and lets you edit the calibration parameters. The effects of any changes you make will be instantly displayed when you close the dialog box, but will not affect the disk copy of the Method unless you resave it. Refer to *Method Editor/Calibration* for a detailed description of the calibration parameters.



Calculator...

Using the fit type and component calibration data specified in the Method, the Calculator can calculate the amount of any component in the Component Table from a known response, or calculate the response from a known amount. Use the scroll bar at the bottom of CalPlot's main window to select the component for which you want to make a calculation.

NOTE: Unlike most dialog boxes, you can use any other CalPlot commands while the Calculator dialog box is open.



Calculate option buttons select either the component Amount or Response for calculation. When Amount is selected, Response is the label for the edit box and the Level list box; when Response is selected, Amount is the label for both boxes.

Type the number on which you want the calculation based into the edit box, then click on the Calculate command button to have the response or amount automatically calculated and displayed. Or, click on the desired value in the Level list box to have that value copied to the edit box and the calculation automatically performed.

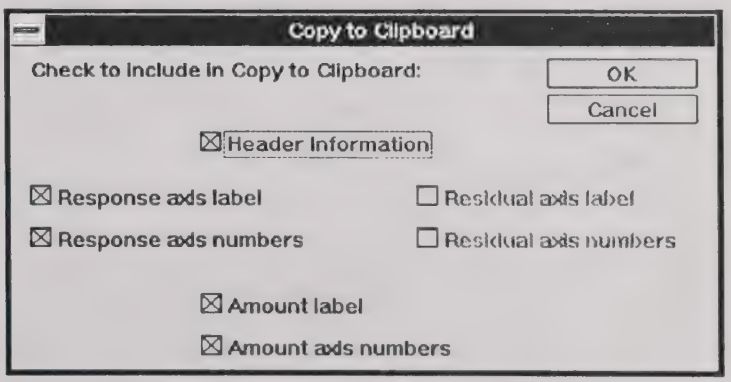
Level displays the amount or response (depending on your selection above) for each calibration level currently stored in the Component Table for the selected component. Up to ten calibration levels can be used. When you click on a value in the list box, it is copied to the edit box and the calculation automatically performed.

Calculate has the selected calculation performed.

Exit closes the dialog box and returns you to CalPlot's main window.

Copy...

Copies the plot currently on view to the Windows Clipboard, from where it can be copied into other Windows-compatible software (for example, into a report created by a desktop publishing program).



To have any of the following items included on the plot copied to the Clipboard, click in the applicable check box.

Header Information includes the information displayed in the box above the calibration plot.

Response axis label includes the word "Response".

Response axis numbers includes the numbers listed along the Response axis.

Residual axis label includes the word "Residual".

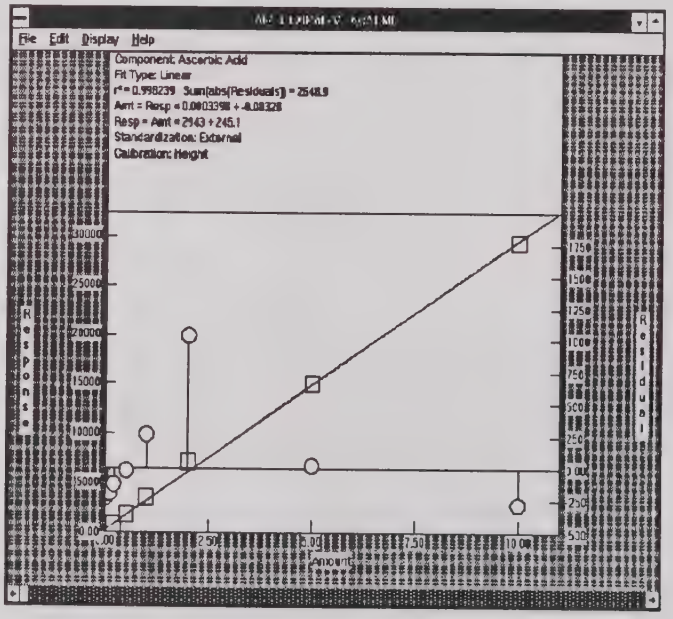
Residual axis numbers includes the numbers listed along the Residual axis.

Amount label includes the words "Amount" and the unit of measurement selected in the Calibration Parameters dialog box.

Amount axis numbers includes the numbers listed along the Amount axis.

Display Menu

There are two display options for plots: Components and Residuals. You can select either or both of these options. The screen below shows both the diagonal Components plot and horizontal Residuals plot for a ten-level calibration of ascorbic acid; the fit type is linear.

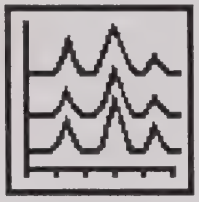


Components

Displays a response vs. amount calibration plot.

Residuals

Plots the difference between the recorded component points and the curve used to approximate the points. A residual plot is useful because it shows you how well the curve fits the data. Changing the fit type and then looking at the resulting residuals plot can help you determine the approximating curve that best fits the component data.If you select this option, the sum of residuals is indicated in the box above the plot.



The View program allows you to recall up to eight stored chromatograms and compare them visually. You can plot each chromatogram on separate axes or overlay them. You can also view a series of chromatograms as a projection along a third axis. The View program does not perform any quantitative calculations.

Starting the View Program

To start the View program, double-click on the View icon in the AI-450 MainMenu or on VIEW.EXE in the directory that holds your AI-450 program files. The View main window will appear. The main window is initially blank, but once you specify which raw data files you want to compare, the chromatograms for each file will be displayed here. The commands, dialog boxes, and windows associated with each View menu are explained on the following pages.

VIEW.EXE Command Summary

File Menu

- Open Raw Data Files...
- Print Plot
- Copy to Clipboard
- Exit

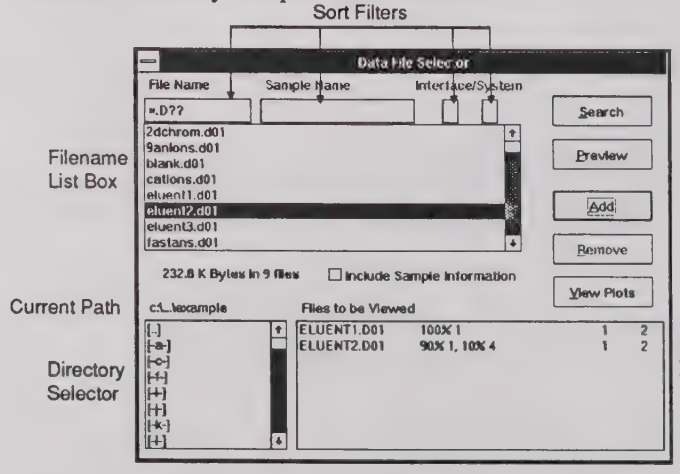
Format Menu
- Separate
- Overlaid
- Projection...
  - Plot Spacing Factor (% of Scale)
  - Angle of Projection (Degrees)
  - Chromatogram Display
    - Transparent
    - Eclipsed
- Scales...
  - Filename
  - Sample Name
  - Start Time
  - Stop Time
  - Range
    - Min
    - Max
  - Use same time for all plots

Options Menu
- Background
  - White
  - Black
- Edit Title...
- Customize Plots...

File Menu

Open Raw Data Files...

The controls in the Data File Selector dialog box allow you to build a list of up to eight data file(s) to be viewed. List boxes within the dialog box allow you to select the desired directory and specific raw data files.



Select Directory Select a directory by clicking on the applicable disk drive and directory in the Directory Selector Box. The Current Path is shown directly above this box.

Select Files Select samples by clicking on the filenames as you scroll through the Filename List Box. To restrict your search to just those samples in which you are interested, enter Sort Filters.

Sort Filters simplify your search for specific samples by filtering out all files which do not match the criteria entered into the Filename, Sample Name, and Interface/System Sort Filters.

Filename Type a filename of from one to eight characters (with no punctuation or spaces) and the extension .D??, where ?? is a number from 01 to 99 and corresponds to the inject number on the schedule. If you leave the Filename List Box blank, the names of all raw data files in the current directory are displayed.

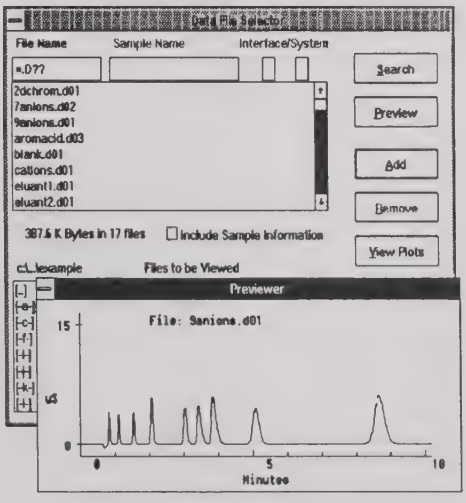
Sample Name Type a sample name of from one to eighteen characters (with no punctuation or spaces).

Interface/System Depending on which model data system you are using, select an Interface, 1 through 6, and system 1 or 2. If you leave these boxes blank, selected files for all interfaces and systems are displayed.

Wild Cards are two special characters, ? and *, that can be used when entering Filename Sort Filters. The ? indicates that any character can occupy that position. The * indicates that any character can occupy that position and all remaining positions in the name, extension, or number field being entered. Wild Cards cannot be used with Sample Names.

Include Sample Information Click on the check box to include sample information for the files. Directory searches are much faster if sample information is not included.

Search After entering any desired Wild Cards, click on the Search button to initiate a search of the current directory for all files that match the Sort Filters. Matching files will be displayed in the Filename List Box.



Preview lets you preview the chromatogram of the data file highlighted in the Filename List Box, as shown here:

Add adds the names of all files that are highlighted in the Filename List Box to the Files to be Viewed Box.

Remove deletes all files that are highlighted in the Files to be Viewed Box.

View Plots displays all of the selected data files, on screen, in the currently selected format and color.

Print Plot

Prints the plots as they appear on the screen.

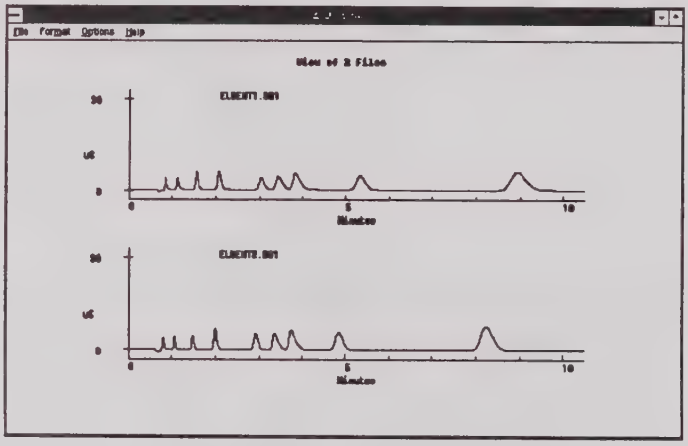
Copy to Clipboard

Copies the displayed chromatograms to the Windows Clipboard. From the clipboard, the chromatograms can be copied into other Windows applications (e.g., Microsoft Write or Aldus PageMaker).

Format Menu

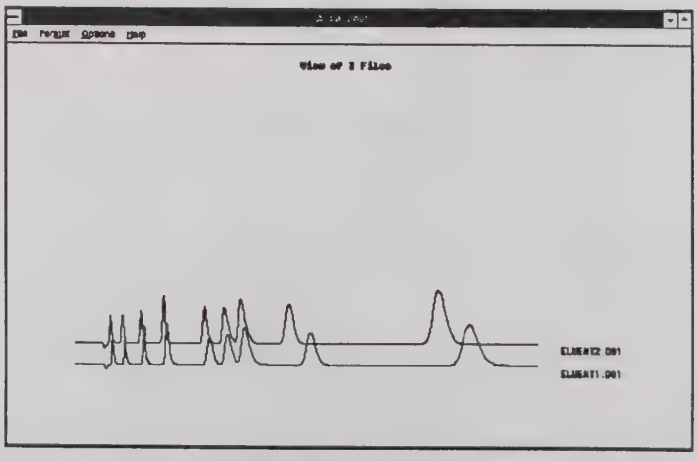
Separate

Displays the selected chromatograms individually, on separate axes, as shown here:



Overlaid

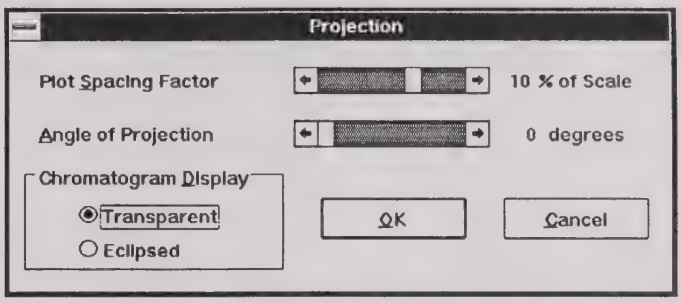
Displays the selected chromatograms on a single set of axes, as shown here:



NOTE: If you want the time axis to be shown on the plot, select the Use same time for all plots option from the Format menu (see *View/Format Menu/Scales...*).

Projection...

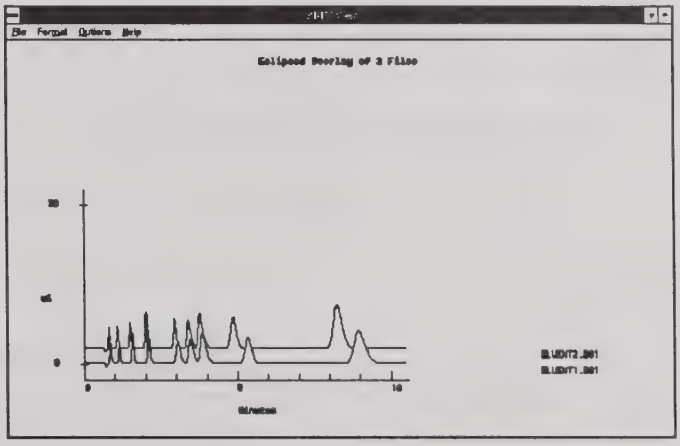
The Projection dialog box lets you display the selected chromatograms as a three-dimensional projection. Projection options apply only to the Overlaid format.



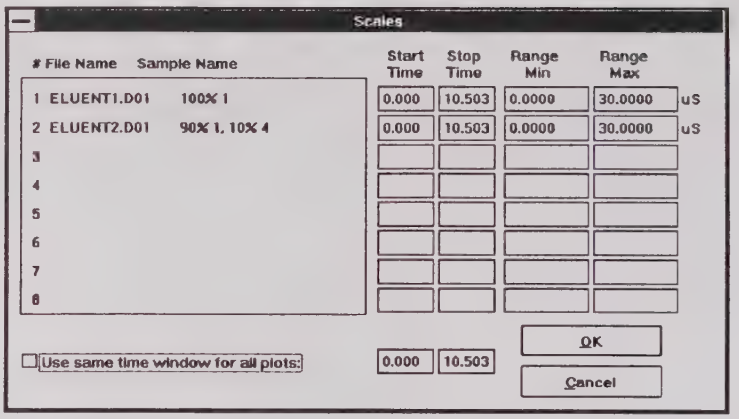
Plot Spacing Factor (% of Scale) sets the spacing between each of the plots along the Y axis of the projection.

Angle of Projection (Degrees) determines how far, in degrees from the vertical, the Z axis of the projection is tilted. This option is applicable only to an Eclipsed Chromatogram Display.

Chromatogram Display Select **Transparent** to have the details of each chromatogram shown. Select **Eclipsed** to have each chromatogram occlude portions of the ones behind and create a three-dimensional effect, as shown here:



Scales...



After you specify the start and stop time of the X axis, and the minimum and maximum range of the Y axis of each plot, the chromatograms are plotted on the screen. If you wish to use the same start and stop time for all plots, select the corresponding check box in the Scales dialog box and enter the desired times in the designated boxes at the bottom of the screen.

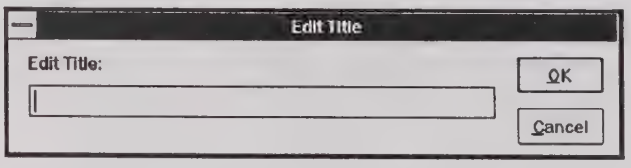
NOTE: If the plot format is Overlaid, the Use same time window for all plots check box must be selected for a time axis to be displayed.

Options Menu

Background

Selects either white or black as the background color of the plot currently displayed.

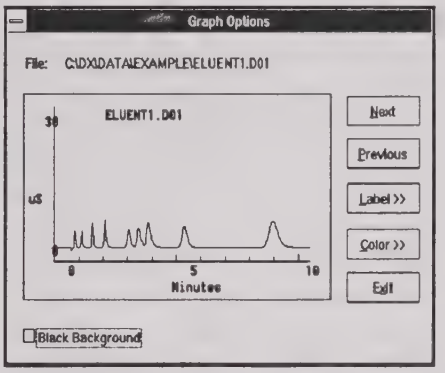
Edit Title...



Enter a title of up to 80 characters in the space provided. (Titles that extend past the end of the edit box will wrap around to the left as you type.)

Customize Plots...

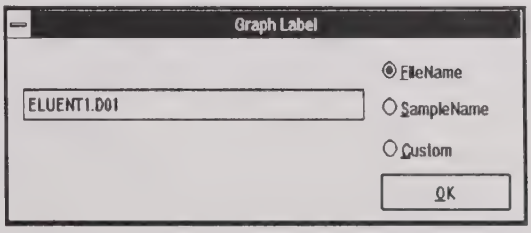
The Graph Options dialog box let you modify the color and name of the data files selected for viewing. A miniature plot of the current file is displayed in the dialog box so that you can preview modifications before accepting them.



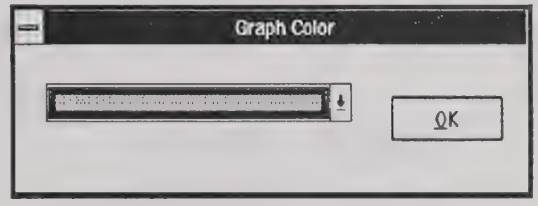
Black Background changes the background color of the plot from white to black.

Next and **Previous** display the next or previous name, respectively, in the list of data files to be viewed. If you selected only one file for viewing, this button is grayed out.

Label >> opens the Graph Label dialog box, superimposing it over the Graph Options dialog box. If you select Filename or Sample Name, the AI-450 automatically adds the name of the file or sample to the window. To create a plot name of your own, enter the name in the space provided, using from one to 40 characters.

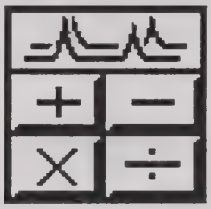


Color >> opens the Graph color dialog box, superimposing it over the Graph Options dialog box. Use the scroll bar to select the color of the plot: white, a pattern, or black.



Exit closes the Graph Options box and returns you to View's main window.

What Is DataMath?



The DataMath program calculates and plots the first and second derivatives of a stored raw data file. It also calculates and plots the mathematical sum, difference, or ratio of any two raw data files. The results of a calculation can be stored as a new data file and used to improve chromatogram quantitation. For example, a baseline data file can be stored and subsequently subtracted from each sample data file. The resulting data file can then be re-integrated, using the Optimize program, to provide more accurate quantitation of detected peaks. DataMath creates and uses the following files:

Files Used	Files Created	Comments
*.Dxx	*.Dxx	Raw data files
*.DAT	*.DAT	DataMath raw data files
	*.DR1	1st Derivative File
	*.DR2	2nd Derivative File

Using DataMath

DATAMATH.EXE Command Summary

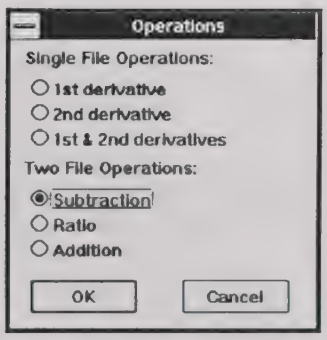
File Menu
Open Data Files...
Save Result Raw Data As...

Edit Menu
Copy to Clipboard

Scales Menu
Scales...

Starting the DataMath Program

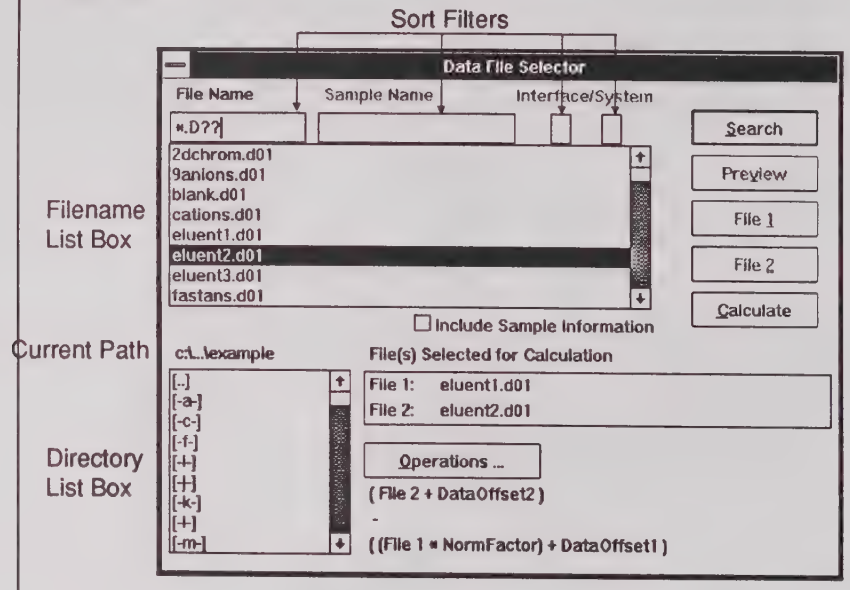
To start the DataMath program, double-click on the DataMath icon in the AI-450 MainMenu or on DATAMATH.EXE in the directory that holds your AI-450 program files. This opens the Operations dialog box:



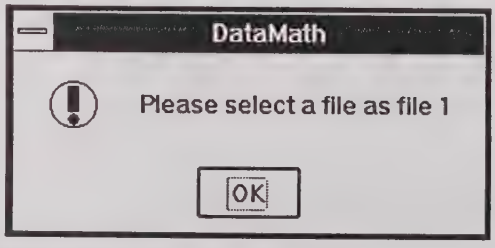
Click the mouse cursor on the mathematical operation(s) you want performed, then click on the OK command button. The Data File Selector dialog box will appear.

Data File Selector Dialog Box

Use the Data File Selector to choose the file(s) on which mathematical operations will be performed. Select just one file if you want to obtain a first, second, or first and second derivative. Select two files if the chosen operation is subtraction, ratio, or addition.



Derivative operations can be performed **only** on original raw data files. If you click on the Calculate command button to calculate a derivative of an existing derivative file, the following message is displayed:



Directory List Box Select the desired directory by clicking on the applicable disk drive and directory.

Filename List Box Select the file you want to open by clicking on the filename. To restrict your search to specific types of data files, enter sort filters.

Sort Filters simplify your search for a specific data file by filtering out all files which do not match the criteria you enter into the Filename, Sample Name, and Interface/System sort filter text boxes.

Filename Type a filename of one to eight characters (no punctuation or spaces) and the extension .D??, where ?? is a number from 01 to 99 corresponding to the inject number in the Schedule. If you leave the Filename list box blank, the names of all raw data files in the currently selected directory are displayed.

Sample Name Type a sample name of one to eighteen characters (no punctuation or spaces). If you type in one or more characters that are the start of a sample name, any samples that begin with the same characters are displayed.

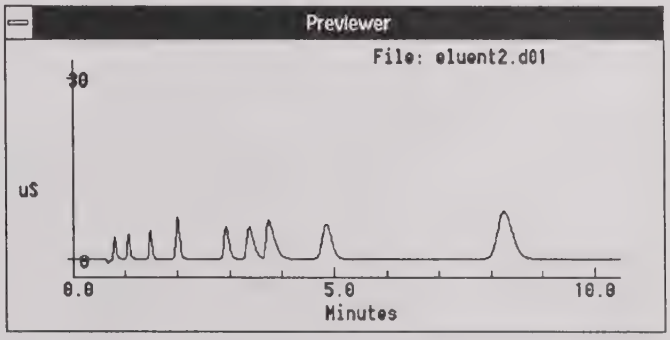
Interface/System Depending on which model data system you are using, select an Interface, 1 through 6, and System 1 or 2. If you leave these boxes blank, selected files for all interfaces and systems are displayed.

Wild Cards are two special characters, ? and *, that can be used when entering a Filename Sort Filter. The ? indicates that any character can occupy that position. The * indicates that any character can occupy that position and all the remaining positions in the name, extension, or number field being entered. Wild cards **cannot** be used with Sample Names.

Include Sample Information Click first on this button and then on the search button to list filenames with their associated sample name, interface, and system information. Listing sample filenames **without** this additional information greatly speeds up a search.

Search initiates a search for files which comply with information entered in the sort filters.

Preview displays a plot (see below) of the sample whose name is highlighted in the Filename list box.



File 1 and File 2 Select only File 1 to obtain a first, second, or first **and** second derivative. Select both a File 1 and a File 2 for a subtraction, ratio, or addition operation.

To select files, first highlight the name of the file in the Filename list box, then click on the File 1 or File 2 command button, as applicable. The selected filename appears in the list box labeled File(s) Selected for Calculation.

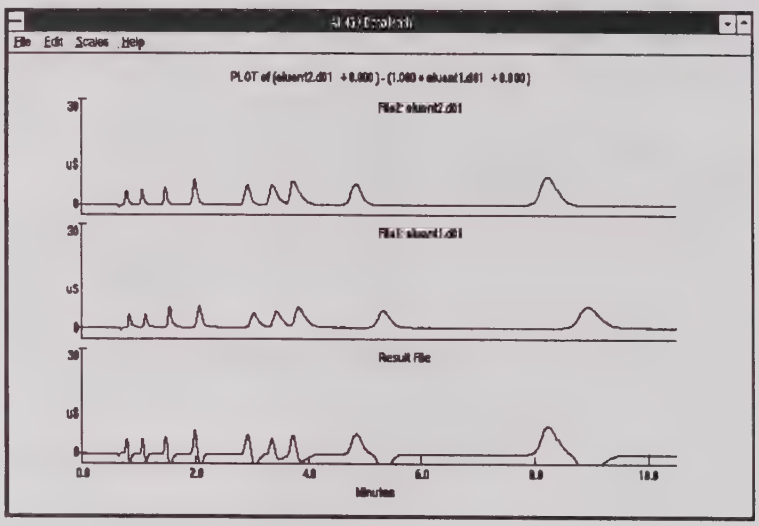
After you select the desired operation, a statement of the mathematical operation to be performed appears at the bottom of the screen. In subtraction operations, File 1 is subtracted from File 2; in ratio operations, File 2 is divided by File 1.

Operations... returns you to the Operations dialog box (see *DataMath/Starting the DataMath Program*). Use this function to request an additional mathematical operation after the one currently requested is completed, or to abort the operation in which you are currently involved.

Calculate begins the mathematical operation. The Data File Selector dialog box disappears. After a brief pause, the plotted calculation is displayed.

DataMath Program Window

The DataMath program window displays the plot resulting from the mathematical operation(s) selected in the Data File Selector dialog box. Three pull-down menus are available in the program window: File, Edit, and Scales.



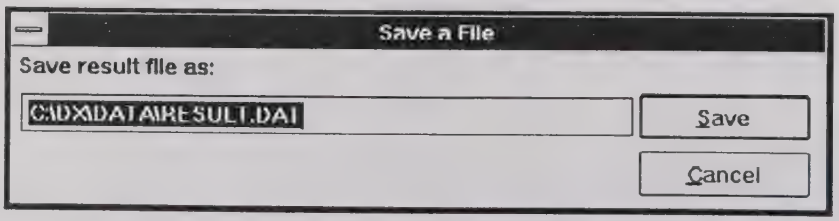
File Menu

Open Data Files returns you to the Data File Selector dialog box. If you want the mathematical operation just completed to be performed on two new files, select the files and then click on the Calculate command button. If you want to **change** the type of mathematical operation, click on the Operations command button to return to the Operations dialog box and make a new selection.

Save Result Raw Data As . . . saves the results of the mathematical operation as a new, separate raw data file. In an operation requiring two raw data files, the parameters used to process the second raw data file are stored with the results. In a mathematical derivative operation, the parameters used to process the first (and only) raw data file are stored with the results.

This command is active only after a mathematical calculation has been performed on the raw data file(s) currently stored in memory. **The result must be saved on disk as a raw data file before it can be processed by the Optimize program.**

Enter a filename of up to eight characters (no punctuation or spaces) for the new raw data file and click on Save. DataMath automatically adds the pathname \DX\DATA and the extension .DAT to the filename. If desired, enter a different path string before the filename. This file can be reprocessed in the same way as any other raw data file.



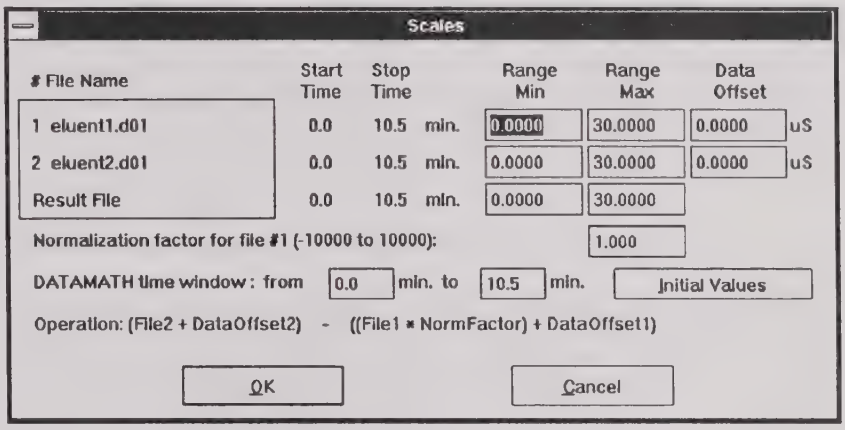
Print Plot prints plots of the original data files and the result of the mathematical operation.

Edit Menu

Copy Plot to Clipboard copies the plots of the original data files and the results of the mathematical operation(s), to the Windows Clipboard. From there, you can copy them into a document written with WRITE.EXE, onto a card in CARDFILE.EXE, or into other Windows-compatible programs. The contents of the Clipboard can be viewed (but not edited) by running the CLIPBOARD.EXE program from the Windows MS-DOS Executive directory.

Scales Menu

The one command on this menu, Scales..., opens the Scales dialog box.



Start Time/Stop Time displays the start and stop times of files 1 and 2 and the start and stop times to be used with the resultant plot.

Range Min/Range Max are the minimum and maximum scale values for the Y axis of each raw data file and for the result file (µS, nS, AU, or mV). The default Range Min value is zero (0); the default Range Max value for files 1 and 2 is the setting specified in the Method used to collect the data. The default Range Max for the result file is the same as the default Range Max value for file 2. Make sure that the scales for each plot are in the same order of magnitude.

Data Offset is the value (µS, nS, AU, or mV) required to compensate for incompatible vertical offsets in the two data files for subtraction, ratio, and addition operations. These values are then subtracted from every data point in the designated chromatogram. This is a useful technique when the baseline of one chromatogram is higher above the zero baseline than the other. If the baselines for both chromatograms are at about the same level, use zero offset (the default value).

Normalization factor for file #1 compensates for differences in sample preparation or size. It is used as a multiplier for the divisor (in the case of a ratio calculation) or the subtrahend (in the case of a difference calculation). A value of 1 is used when there are no differences. The default value is 1.

For example, to find the ratio of chromatogram A to chromatogram B (A/B) using a normalization factor of 1.5, the actual ratio of every data point is calculated as:

(chromatogram A)/(1.5 X chromatogram B)

Similarly, if you want to subtract chromatogram A from chromatogram B (B-A) using a normalization factor of 2.3, the actual difference is calculated as:

(chromatogram B) - (2.3 X chromatogram A)

DataMath time window indicates the start and stop times. The start time default value is zero (0). The stop time default value is the **shorter** of the two stop times originally stored with the raw data files. Clicking on the Initial Values >> command button returns all values in the Scales dialog box to default values.

Initial Values resets all values to those originally saved with the file.

Operation displays the operation performed on the plots for which parameters are being selected.

OK modifies the plot(s) in accordance with the parameters selected in the dialog box.

Cancel cancels any parameter changes made in the Scales dialog box and returns to the plot display.

NOTE: An asterisk precedes any term, or usage of a term, specific to Dionex.

ADDRESS, GPIB DEVICE: The unique address assigned to each GPIB-equipped device (e.g., an Advanced Computer Interface) which is connected to the host computer. This address allows the computer to specify to which device it is talking/listening. The GPIB addresses used by the ACI must match the GPIB address assigned in the GPIB.INI file.

***ADVANCED COMPUTER INTERFACE (ACI):** The Dionex module that provides the communications and control link between the AI-450 and other Dionex modules or other analytical instruments. The AI-450 sends commands to the ACI as strings of ASCII characters via either an RS-232C or a GPIB (IEEE-488) interface. When the ACI microprocessor receives a command from the AI-450, it translates the ASCII characters into binary instructions used to control the attached modules. Conversely, the ACI translates binary data received from the attached modules into ASCII and transmits them to the AI-450.

ANALOG DATA: Non-digitized output from a detector.

BUNCHING: The process of averaging a number of digitized data points to form a single bunch for use by the software. Bunching improves the signal-to-noise ratio by allowing a larger data sample to be averaged. By adjusting the number of data samples in a bunch, the resulting filtering can be tailored to the requirements of the peaks in an individual chromatogram.

BUNCHING FACTOR: The number of digitized data points being averaged in order to create a single data point for data reduction.

CALIBRATION CURVE: A mathematical equation that relates detector response in peak height or area versus amount.

CALIBRATION LEVEL: A set of values for concentration, area, and height that are used to calibrate a Method. A single-level calibration assumes a linear detector response that passes through zero over the expected amount range of each component in the sample. If the detector response is non- linear or does not pass through zero, it is necessary to use several calibration standards covering the expected amount range. The data for each standard is then used to determine a calibration curve for each sample component.

CHANNEL: See **SYSTEM**.

DATA, RAW: A digitized chromatogram which has not been processed to detect and identify peaks and determine their heights and/or areas. Raw data files include the extension ".Dxx", where xx is a number from 01 to 99. See **RAW DATA FILE**.

DATA REDUCTION: The transformation of raw data into intelligible form by averaging, adjusting, scaling, ordering, and other statistical methods. The data reduction parameters set in the method are used by the Run program to determine baselines, peak retention times, peak heights and/or areas, peak identification, and perform quantitation.

DEFAULT: Preprogrammed settings and parameters that are used when no other settings are selected by the user.

DIGITIZING: For a computer to process a chromatogram, the detector response must be converted from an analog (continuous) signal to a digital (discrete) signal. The resolution of the Analog-to-Digital (A/D) converter and the frequency of data sampling determine the accuracy and precision with which peaks can be measured. Several Dionex detectors, such as the CDM-II, VDM-II, PED, and CES detectors, have built-in A/D converters, but most detectors produce only an analog signal that must be converted to digital format by the high-resolution A/D converters in the Advanced Computer Interface.

DIRECTORY: A DOS tool for grouping and organizing files on a disk. See **ROOT DIRECTORY**.

DRIVE SPECIFIER: A letter followed by a colon, which refers to a disk drive (e.g., C: for the first hard disk).

EFFICIENCY (N): A measure of peak dispersion as it moves through a column. Efficiency is proportional to column length: for a given stationary phase and column diameter, increasing the column length increases the efficiency. Synonymous with **THEORETICAL PLATES**.

$$N = 5.54(t_1/w_{1/2})^2$$

where:

t_1 = the peak retention time
$W_{1/2}$ = the peak width at 1/2 height

ERROR: Any deviation of a computed or a measured quantity from the theoretically correct or true value. In a computer system, an error arises when the system receives information to process or instructions to execute which it does not recognize. Computers are precise, literal machines which require very precise instructions and specific formats. An error condition arises when an instruction or data does not match the expected format.

EXTERNAL STANDARD An analytical method of quantitation which uses calibration standards containing known amounts of constituent compounds to calibrate the system. The measured response of each component is then used to determine the presence and concentration of the same components in samples of unknown composition. This technique compensates the non-uniform detector response to the sample constituents. Use of an external standard assumes that the injection volumes for both the calibration standards and the actual samples are known.

FILE: A set of information stored on disk.

FUNCTION KEY: A programmable function key on a computer keyboard. Pressing a function key triggers automatic execution of whatever operation the key controls.

GPIB (IEEE-488) INTERFACE: An industry-standard parallel interface. It allows the host computer to communicate with as many as sixteen different GPIB-equipped devices (e.g., a disk drive, a printer, and up to ten ACIs) along a single wire or bus. Each device connected to the bus is assigned a unique address which distinguishes it from the others.

HARD DISK: A device for non-volatile, random-access data storage. Hard disks have a larger storage capacity, faster access, and tend to be more reliable than diskettes. Hard disks are encased in dust- free enclosures.

INTEGRATION A process which calculates the area under a peak.

INTERNAL STANDARD: An analytical method of quantitation in which a known concentration of a non-interfering compound is added to each sample. The measured response of this compound is then used to calculate the concentration of the components in samples of unknown composition. This technique compensates for variations in sample size. Use of the internal standard method assumes a uniform response for all of the sample constituents.

MASS ON COLUMN: The mass of the injected component. The calculation is shown below:

$$\text{Mass on Column} = \text{Injection Volume} \times \text{Concentration}$$

NOISE: Unwanted electrical disturbances caused by very low-level, random electrical activity. This electrical activity is present at all times, even in the absence of useful data signals.

NORMALIZED AMOUNT: A value indicating the percentage ratio of the amount of a component to the sum of all components. The calculation is shown below:

$$\text{Normalized Amount}_i = \left(\frac{\text{Amount}_i}{\sum_{i=1}^{N} (\text{Amount}_i)} \right) \times 100$$

Where:

Amount_i = the calculated amount of component_i

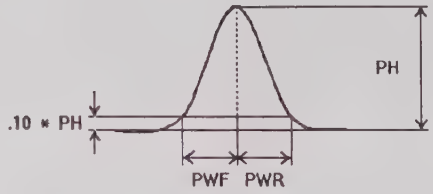
OFFSET: A value, usually a voltage or numeric constant, which is added to another value, usually a changing signal voltage or its mathematical representation. The offset ensures that a negative value does not occur. For example, a signal with a range from -2V to +2V may have a +2V offset added at all times, so that the voltage range will be from 0V to +4V.

***PARALLEL REMOTE:** The 50-line parallel interface used in the ACI. This interface provides for remote control and monitoring of suitably equipped Dionex modules.

PATH STRING: The list of nested subdirectories, starting with the root directory, which leads to a specific filename or subdirectory.

PEAK: The maximum value of a continuously varying quantity during a specified period. A chromatographic peak traces the varying concentration of a compound as it elutes from the end of the separator column.

PEAK ASYMMETRY: A measure of the fronting or tailing of a peak. The calculation is shown below:



Asymmetry = PWR/PWF

Where:

PH	=	the peak height
PWF	=	the peak width front measured at 10% of peak height above baseline
PWR	=	the peak width rear measured at 10% of peak height above baseline

PEAK THRESHOLD: The minimum positive slope which indicates the beginning of a peak; measured in counts-per- second. The peak threshold is a measure of how fast the leading edge of the peak must rise before it is recognized as a peak. The larger the number (slope), the sharper the peak must be.

PEAK WIDTH: The peak width at half-height of the narrowest peak in the chromatogram. The peak width determines the extent of bunching used by the peak detection algorithm.

POLLING RATE: A measure of how frequently the computer checks the status of the ACI and retrieves data.

RAW DATA FILE: The raw data file contains all of the digitized data points collected during an analysis. It also includes all parameters of the Method originally used to process the data. The extension ".Dxx", where xx is a number from 01 to 99, is automatically added to the data filename when the data is stored on disk. The number in the extension represents the iteration of the Method or the line number in the Schedule (e.g., RAWDATA.D01, RAWDATA.D02, etc.).

REFERENCE COMPONENT: A component within a chromatogram whose retention time is used to correct for variations in the actual retention time of another component.

***RELAY CONTROL:** A control mode supported by Dionex modules. Relay Control uses simple TTL logic levels or relay contact-closures to control selected module functions. All of the remaining functions are controlled using the switches on the module front panel.

REPORT FILE: An ASCII file that includes the retention times, peak names, peak areas, peak heights, and calculated concentrations for every peak included in the report. The result data is stored under the same filename used for the raw data but with the extension ".Rxx", where xx is a number from 01 to 99.

APPENDIX B - PRINCIPLES OF PEAK INTEGRATION

Data Samples

Chromatographic detectors generate an analog signal which varies with changes in the composition of the materials passing through the detector cell. The analog signal is then digitized, either by circuitry in the detector or the Advanced Computer Interface (ACI), to produce a voltage/time integral called a **data sample**. This integration tends to average or "filter out" high frequency noise. The ACI digitizes data from two detectors at once. The ACI sampling rate can be set to 1, 2, 5, 10, 20, or 50 samples per second (Hz).

Sampling Rate

The **sampling rate** determines the number of data points per second collected and stored in the ACI memory. Increasing the sampling rate (i.e., selecting a larger number of samples per second) increases the accuracy of detecting and integrating very fast peaks by increasing the number of data points collected in each sampling period. It is usually best to choose a sampling rate that defines the narrowest peak with 20-30 data points.

The sampling rate also affects the amount of data that can be stored in the ACI memory. Increasing the sampling rate decreases the length of time that the ACI can store data before that data must be retrieved by the computer. Faster sampling rates also consume more disk space and slow down reprocessing of stored data.

The effect of the sampling rate on the amount of data that can be stored depends on whether your ACI has a storage capacity of 256K or 64K per system. Because of the data compression routine used by the ACI, the type of data being collected also affects the length of time that data can be stored. Under conditions in which the detector output is constantly changing, more bits of data are stored and memory is filled more quickly than when the output changes less frequently (the values for ACI Minimum Capacity in the two tables that follow). Conversely, when the detector output remains relatively constant (i.e., a baseline), data with fewer bits are stored and memory fills more slowly (the values for ACI Maximum Capacity value).

256K Advanced Computer Interface (Shipped after June 1, 1992)

For a system with only one detector, an ACI with 256k of memory per system will hold approximately 6 hours of raw data at 5 samples/second; at 50 samples/second, it will hold approximately 34 minutes. Adding a second detector to the system reduces these times by approximately half.

Peak Width (Seconds)	Sampling Rate (Hz)	ACI Capacity with One Detector (Minutes)		
		Minimum	Typical	Maximum
> 10.0	1	840	1680	4200
5.0 - 10.0	2	840	420	2100
2.0 - 5.0	5	168	336	840
1.0 - 2.0	10	84	168	420
0.5 - 1.0	20	46	84	210
< 0.5	50	16.8	33.6	84

64K Advanced Computer Interface (Shipped before June 1, 1992)

For a system with only one detector, an ACI with 64k of memory per system will hold approximately 1.5 hours of raw data at 5 samples/second; at 50 samples/second, it will hold approximately 8 minutes. Adding a second detector to the system reduces these times by approximately half.

Peak Width (Seconds)	Sampling Rate (Hz)	ACI Capacity with One Detector (Minutes) Minimum	Typical	Maximum
> 10.0	1	210	420	1050
5.0 - 10.0	2	210	105	525
2.0 - 5.0	5	42	84	210
1.0 - 2.0	10	21	42	105
0.5 - 1.0	20	11.5	21	52.5
< 0.5	50	4.2	8.4	21

NOTE: You can expand your ACI's memory to 256K per system with the optional RAM upgrade kit (P/N 043592).

Data Bunching

After the raw, digitized data is collected and transmitted to the computer, the software sums groups of data samples into **data bunches**. Averaging a larger number of samples improves the signal-to-noise ratio. By adjusting the number of data samples in a bunch, (the **bunching factor**), the resulting filtering can be tailored to the requirements of the peaks in an individual chromatogram.

The bunching factor is calculated from the minimum peak width and "time per sample" (the inverse of the sampling rate) selected in the Method (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Integration*). The AI-450 software sums ten data bunches per peak width value.

$$\textit{Bunching Factor} = \frac{\textit{Minimum Peak Width}}{10 \times (\textit{Time per Sample})} = \frac{\textit{PW} \times (\textit{Sampling Rate})}{10}$$

If the bunching factor is 1, no values are averaged, and the software considers every raw data point in its evaluation of peaks and areas. If the bunching factor is 5, the software averages every 5 consecutive raw data points to form a bunched data point.

NOTE: Bunched data points are not displayed.

The optimum bunching factor can be determined by measuring the baseline width, in seconds, of the narrowest peak in the chromatogram. This peak width parameter is specified in the Method (see *Method Editor/Data Processing Parameters/Integration*) and, along with the sampling rate, establishes the initial bunching factor. Using a higher factor screens out more noise. However, too high a bunching feature may cause small peaks to be missed in the analysis.

In general, very narrow peaks require a very small bunching factor (such as 1) to prevent them from being treated as noise. Conversely, very broad peaks must have a relatively large number of data samples per bunch to prevent them from being treated as baseline drift.

Late-eluting peaks in a chromatogram are typically broader than peaks that elute earlier, but if there is a significant difference it may be necessary to increase the bunching factor during the run so that the broadest peaks are detected as peaks and not as baseline drift. This can be done by including one or more data timed events in your Method to change the bunching factor (see *Method Editor/Data Processing Parameters/Detector 1 or Detector 2/Data Events*). Changing the bunching factor optimizes the number of averaged data samples used for peak detection and integration throughout the entire chromatogram. For example, doubling the bunching factor causes twice as many raw data samples to be averaged when detecting low, broad peaks.

Peak Detection

The AI-450 software uses slope, or rate of change in signal level, to detect peaks. A new slope is calculated each time a new data bunch is formed. Thus, the peak width parameter in your Method also determines how frequently the slope is calculated. Narrow peaks require small bunch sizes so that the slope is calculated frequently enough to detect and confirm a peak. Wide peaks, on the other hand, require larger bunches so that a more gradual change in the signal level is detected as a peak.

Peak detection is activated when the measured slope of the peak exceeds the peak threshold selected in the method (see *Method Editor/Data Processing Parameters/Integration Parameters*). The peak threshold is a measure of how much the leading edge of the peak must rise before it is recognized as a peak. The larger the number (slope), the sharper the peak must be. When the data exhibits a slope greater than this threshold, the first data point is marked as a potential beginning of a peak. A peak is confirmed if the slope continues positively until the accumulated area is 100 times the peak threshold (Figure B-1).

After confirming the beginning of a peak, the software begins searching for the peak crest. The crest is indicated when the signal reaches a maximum and then steadily declines from that maximum until the accumulated area is 100 times the peak threshold value. Finally, the software searches for the end of the peak. The end of the peak is detected when the slope fails to decline by the peak threshold value for three consecutive data bunches.

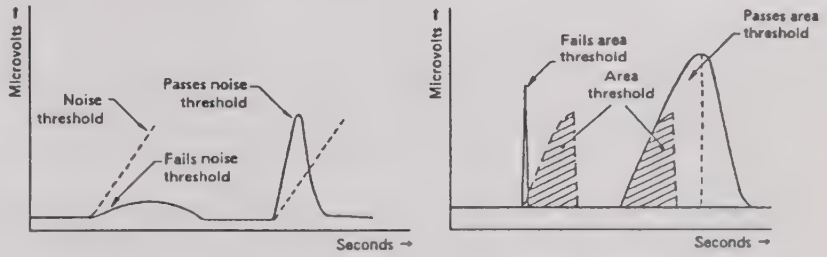


Figure B-1 *The Effect of Peak Threshold and Area Threshold on Peak Detection*

Peak Integration

After completing peak detection, the software evaluates the detected peaks and places each peak in one of four baseline categories. These categories in turn determine how the baseline for each peak is calculated.

Peak Type	Category
Completely resolved	1
Partially resolved, similar size	2
Mother peak	3
Shoulder peak	4

Peaks in the first baseline category (BL=1) are completely resolved. The baseline for this type of peak is a line between the beginning and ending points of the peak.

Peaks in the second baseline category (BL=2) are incompletely resolved peaks of similar size. These peaks are separated by a drop-line extending from the valley between the peaks to a common baseline.

Peaks in the third and fourth baseline categories are adjacent peaks which meet the following requirements: the first peak in the pair (BL=3) must have a closely tailing shoulder peak (BL=4) that has less than 25% of the area or height of the first peak. These peaks are separated by an exponential projection of the peak shapes. Peaks which do not meet **both** criteria fall into the second baseline category and are separated by a drop-line.

Peaks are assumed to be overlapped if a neighboring peak begins within 25% of the width of the previous peak. Overlapped peaks are given a common baseline extending from the start of the first peak to the end of the last peak in the cluster. Intervening valleys are then scanned to detect baseline penetration. If baseline penetration is detected, the end of the baseline for the cluster is moved to the point of penetration.

Finally, the software determines whether to separate clustered peaks by drop-lines or by exponential skimming. With the baseline established, the software then integrates the data bunches under the peak to determine the area. The peak areas or heights are then used to calculate amounts (Appendix C).

APPENDIX C - QUANTITATION TECHNIQUES

After raw data from an analysis is acquired, algorithms which use the parameters set in the Method (e.g., peak threshold and time window) perform peak detection, retention time determination, and peak area calculations. From this basic information, the data can be further subjected to quantitation techniques to express it in terms of concentrations. All of these calculations occur after the raw data is transferred to the computer from the Advanced Computer Interface (ACI).

The selection of a quantitation method should be based on its applicability to the sample material and the accuracy required. The quantitation methods discussed in Appendix C are those most frequently used today. Specifying a particular technique not only causes the data to be processed in a particular way, but also imposes certain restraints on the experimental procedure.

Response Factors

The response of any given detector or detector type varies from one chemical compound to another. Although some classes of compounds may have similar detector responses, it is equally possible that isomers of the same chemical may have very different detector responses. This difference in detector response is the primary reason that it is necessary to calibrate your analytical system; temperature drift and column degradation are additional factors.

Part of the calibration process is to calculate response factors, which are a measure of the relative detector response for each component. The Run and Batch programs automatically calculate a response factor for each component in the calibration standard whenever you specify either an external or internal standard report in your Method. Each time you repeat a calibration run for a particular analysis, these factors are updated, either by averaging the new value with the previously stored value or by replacing the old value with the new one.

External Standard Quantitation

The external standard quantitation technique is used for analyses where the detector response for each component in the sample is different and independent of other components. When using external standards, the amount of each sample component is calculated with respect to the absolute response for that component.

External standard quantitation is also quite useful for trace analyses, where the error inherent in repeated sample injections is not critical. The use of external standard quantitation is especially attractive for use with an autosampler, where repetitive injection volumes are reproducible.

Since each response factor is independent of other components, response factors are needed only for components of interest. The presence of other components does not affect quantitative results, and not all components need be eluted or detected.

There are a few requirements for external standard quantitation:

- The composition of the calibration mixture must be close to that anticipated for samples.
- Because this technique is dependent on sample size, the injection volumes for each calibration run and sample run must be known, or at least be reproducible.
- Calibration should be repeated frequently to compensate for drift in the chromatographic system.

External Standard Calculations

NOTE: Peak height can be substituted for peak area in the following equations if you are quantitating by peak height in your Method.

For a single-level calibration, the absolute response factor for each component, i, is calculated as:

$$ARF_i = Amt_i / Area_i$$

Where:

ARF_i	=	the absolute response factor for component i
Amt_i	=	the amount of component i in the calibration standard
$Area_i$	=	the area of the component i in the calibration standard

After the response factors for all the components in the calibration standard have been determined, the sample can be injected and the response-corrected concentrations of the components calculated as:

$$Amt_i = (Area_i)(ARF_i)(MulFactor)$$

Where:

Amt_i	=	the amount of component i in the sample
$Area_i$	=	the area of component i in the sample
ARF_i	=	the response factor for component i
Mul Factor	=	the multiplication factor

Internal Standard Quantitation

Like external standard quantitation, the internal standard quantitation technique is used for analyses where the detector response for each sample component is different. Because quantitation of an unknown peak is relative to the internal standard, the analysis is independent of variations in detector sensitivity, sample size, and other sample components.

Internal standard quantitation involves adding a known amount of a reference compound to each injection of the calibration standard and sample. This reference must elute in an open area of the chromatogram and must not interfere or chemically interact with any of the sample components. It should also exhibit chromatographic properties similar to those of the unknown component(s) to which it is compared.

Internal standard calculation allows the accurate quantitation of individual components without regard to changes in chromatographic conditions or injection volume. Ideally, the amount of the internal standard component should be slightly larger than that of the peaks of interest. For best results, the area of the internal standard component should not vary by more than a factor of 10 from the areas of the peaks of interest.

Internal Standard Calculations

NOTE: Peak height can be substituted for peak area in each of the following equations if you are quantitating by peak height in your Method.

For a single-level calibration, the relative response factor for each component, i, is calculated as:

$$RRF_i = \frac{(Amt_i)/(Area_i)}{(Amt_{ISTD})/(Area_{ISTD})}$$

Where:

RRF_i	=	the relative response factor for component i
Amt_i	=	the amount of component i in the calibration standard
$Area_i$	=	the area of the component i peak
Amt_{ISTD}	=	the amount of the internal standard in the calibration standard
$Area_{ISTD}$	=	the area of the internal standard component

After the relative response factors for all of the components in the calibration standard have been determined, the sample can be injected and the response-corrected amounts of the components calculated as:

$$Amt_i = \frac{Area_i}{Area_{ISTD}} \times Amt_{ISTD} \times RRF_i \times Mul\ Factor$$

Where:

Amt_i	=	the amount of component i in the sample
$Area_i$	=	the area of component i in the sample
Amt_{ISTD}	=	the amount of the internal standard in the sample
$Area_{ISTD}$	=	the area of the internal standard peak in the sample
RRF_i	=	the response factor for component i
Mul Factor	=	the multiplication factor

Multilevel Calibrations

Single-level calibrations assume a linear detector response that passes through zero over the expected concentration range of each sample component. If detector response is non-linear or does not pass through zero, it is necessary to use a multilevel calibration. This means that several calibration standards covering the expected amount range are injected. A calibration curve for each component is determined from the data acquired for each calibration level, using either a linear, point-to-point, quadratic, or cubic interpolation.

After the calibration curves are determined, the amounts of the components in the sample mixture can be calculated by relating the measured area to its corresponding calibration curve.

- When a linear fit is used, the calibration curve is determined by a least-squares calculation to fit a line through the calibration data points.
- When a point-to-point fit is used, the software determines which two calibration data points bracket the response of the unknown and uses a straight line between the two points to interpolate the concentration of the unknown.

- When a quadratic fit is used, the calibration curve is drawn from a quadratic equation. A quadratic fit is most useful when the detector response is known to "flatten out" toward one extreme or the other of the concentration range.
- A cubic fit is useful for curves that have both positive and negative inflections, such as electrochemical curves.

Curve Fits

The AI-450 software supports linear, point-to-point, quadratic, and cubic curve fits. Each of these curve fitting procedures is described below.

Linear Curve Fit

The linear curve fit models the detector response as a linear response factor. The response line does not assume a pass through the origin (zero), except with a single-level calibration or unless the force zero option is selected.

The linear curve fit is also known as the least squares, or linear regression, technique. It can be used whenever the detector response is approximately linear, and when the assumption of a linear response does not create a significant error. It can also be used for a narrow concentration range over which a somewhat linear detector response exists, or can be assumed, without producing substantial error.

This curve fit computes a relative or absolute response factor for each calibrated component in a calibration. The software then constructs a straight-line equation that best fits the response versus amount data points over all the levels. In a single-level calibration, this line is between the origin (0,0) and the calibration data point for the component. The response factor (amt/response) is then used in conjunction with the selected calibration formula to report the amount or concentration of each component in the sample.

The straight-line equation computes the response factor, Y, from the general straight-line equation in the slope-intercept form:

$$Y = K_0 + K_1 \times X$$

Where:

K_0	=	the Y-axis intercept
K_1	=	the slope of the response line

The response factor computed depends upon the calculation procedure selected. For external standard calibrations, an absolute response factor is computed. For internal standard calibrations, a relative response factor is derived.

For external standard calibrations, the slope, K_1, is computed according to the following formula:

$$K_1 = \frac{\sum_{j=1}^{n} [(CA)_j (Amt)_j] - \frac{[\sum_{j=1}^{n} (CA)_j][\sum_{j=1}^{n} (Amt)_j]}{n}}{\sum_{j=1}^{n} [(CA)_j]^2 - \frac{[\sum_{j=1}^{n} (CA)_j]^2}{n}}$$

Where:

$(Amt)_j$	=	the amount of a component in the calibration standard at level n
$(CA)_j$	=	the calibrated response (area or height) for a component at level n
n	=	the number of levels in which a component is calibrated

The Y-axis intercept, K_0, is computed according to the formula:

$$K_0 = \frac{\sum_{j=1}^{n} [(Amt)_j] - m [\sum_{j=1}^{n} (CA)_j]}{n}$$

For internal standard calibrations, the slope, K_1, is computed according to the following formula:

$$K_1 = \frac{\sum_{j=1}^{n} [\frac{(CA)_j}{(CA)_{ISTD}} \frac{(Amt)_j}{(Amt)_{ISTD}}] - \frac{\sum_{j=1}^{n} [\frac{(CA)_j}{(CA)_{ISTD}}] \cdot [\sum_{j=1}^{n} \frac{(Amt)_j}{(Amt)_{ISTD}}]}{n}}{\sum_{j=1}^{n} [\frac{(CA)_j}{(CA)_{ISTD}}]^2 - \frac{\sum_{j=1}^{n} [\frac{(CA)_j}{(CA)_{ISTD}}]^2}{n}}$$

Where:

$(Amt)_j$	=	the amount of a component in the calibration standard for level n
$(CA)_j$	=	the calibrated response (area or height) for a component
$(CA)_{ISTD}$	=	the calibrated response (area or height) of the internal standard component
$(Amt)_{ISTD}$	=	the amount of the internal standard component
n	=	the number of levels in which a component is calibrated

The y-intercept, K_0, is then computed as:

$$K_0 = \frac{\sum_{j=1}^{n} \left[\frac{(Amt)_j}{(Amt)_{ISTD}}\right] - K_1 \left[\sum_{j=1}^{n} \left[\frac{(CA)_j}{(CA)_{ISTD}}\right]\right]}{n}$$

Each component must be calibrated over at least two levels in order for this curve fit to work. If fewer than two levels exist, the calibration curve is forced through the origin (zero).

Forcing a non-linear function to be linear should only be done when the resulting error produced would be minimal. This procedure can also be used when calibration is performed within a range where response is most linear.

When using an internal standard procedure, an internal standard component must be calibrated in each level.

Point-to-Point Curve Fit

A point-to-point fit is often used to match an arbitrary response curve with the range of the identified calibration amounts. The segment of the response below the lowest calibrated amount is assumed to pass through the origin (zero). Therefore, it is a good practice to calibrate at a point below the lowest expected sample amounts for any samples whose response curves do not pass through the origin or are unknown.

The segment above the highest calibrated amount is assumed to be a linear extrapolation of the segment between the second highest and highest calibrated amounts. If the actual response above the highest level is unknown or does not follow this behavior, make sure that the highest calibrated amount is greater than any expected sample amounts.

The formula used to compute a point-to-point calibration is:

$$Y = \frac{Y_2 - Y_1}{X_2 - X_1} (X - X_1) + Y_1$$

Where:

- Y_2 = the calibrated amount of the component in the next level
- Y_1 = the calibrated amount of the component in the present level
- X_2 = the calibrated response of the component in the next level
- X_1 = the calibrated response of the component in the present level

The response factor, K_1, is then computed from the following:

$$K_1 = \frac{Y_2 - Y_1}{X_2 - X_1}$$

The y-intercept, K_0, is then computed as:

$$K_0 = Y_1 - K_1 \times X_1$$

If the response function is a second-order model, the quadratic fit method may be more desirable since it produces a more accurate response factor.

Non-Linear, Quadratic Curve Fit

The non-linear curve fit produces a response factor from a quadratic equation of parabola that best fits a detector's second-order response. The general quadratic equation used to compute the response factor for each component in a multilevel calibration is:

$$Y = K_0 + K_1 \times X + K_2 \times X^2$$

The final equation produces the relative, or absolute, response factor, which is then used to compute the amount of the component according to the selected calibration formula.

Because at least three points are needed to fit a parabola, the calibration must have at least three levels for all components. When using an internal standard calibration, an internal standard component must be calibrated in each level.

Non-Linear, Cubic Curve Fit

The response factor produced from a cubic equation best fits a detector's third-order response. The equation used to calculate the response factor is:

$$Y = K_0 + K_1 \times X + K_2 \times X^2 + K_3 \times X^3$$

Because at least four points are needed to fit the equation, the calibration must have at least four levels for all components.

APPENDIX D - TROUBLESHOOTING THE HARDWARE INSTALLATION

This troubleshooting section is intended to help isolate and solve problems related to hardware installation. It is not intended to help troubleshoot Methods development.

The most common source of installation problems are oversights or errors in connecting cables. Refer to the *Advanced Computer Interface Installation Manual* (Doc. No. 034011) for additional information.

One or More Modules Not Controlled Correctly

1. Make sure that the Advanced Computer Interface (ACI) is configured correctly for the components in your system, as instructed in the chapter entitled *Setting Up Your System* in the AI-450 manual.
2. Make sure that the 50-pin parallel cables are connected correctly.

No Data Collected

1. Make sure the detectors are correctly connected to the ACI.
2. Make sure that "Begin Sampling" is included in the Timed Events.
3. Make sure that data stored in the ACI RAM is transferred to the computer before it "wraps around". See Appendix B for detailed information about the amount of data that can be stored in the ACI memory before it is transferred to the computer.

No Data Collected from an Analog Detector

1. Make sure that the cable connections to the DET connectors on the ACI rear panel are installed correctly.
2. Make sure that the correct DET connector was selected in the Method.

No Relay or AC Control

1. Use Dionex relay cables (P/N 041558) to make connections to the RELAY connectors on the ACI rear panel.
2. Make sure that a power source is connected to the AUX AC connector on the ACI rear panel and that it matches the power requirements of the accessories that will be controlled by the AC outlets.
3. Make sure that the AUX AC fuse on the ACI rear panel is not blown.
4. Make sure that the Timed Events turn on the relay or AC outlet.

APPENDIX E - USING DOS

Before installing the AI-450 Chromatography Software on your computer, create a DOS (Disk Operating System) directory on your hard disk (e.g., C:\DOS) and copy all of the DOS Utilities into it. Failure to do this will prevent the AI-450 software from using virtual memory.

DOS is basically a file management program which keeps your files sorted so that you (and your programs) can find them efficiently. It also includes all of the utilities needed to copy, delete, rename, and otherwise manipulate your files.

NOTE: If you have an IBM computer, use PC-DOS. The function of PC-DOS is virtually identical to MS-DOS, except that it includes a version of BASIC that works only on the IBM machine.

The Windows Operating Environment, under which the AI-450 software runs, operates on top of DOS. Many of the commonly used DOS utilities are accessed through the pull-down menus in the Windows File Manager.

This appendix describes briefly the use of directories and pathnames for organizing information on your disks. For additional information about MS-DOS, refer to any of the readily available books on the subject. One that is particularly good is *MS-DOS User's Guide*, 2nd Edition, by Chris DeVoney (Indianapolis: QUE Corporation, 1987).

Files

Computers store information on magnetic disks in groups called files. A file can contain text, a picture, program instructions, or digitized detector data. DOS has the following rules for naming files:

- A filename may contain letters and/or numbers
- A filename consists of two parts, a filename and an extension, that are separated by a period (or "dot"). The filename can be up to eight characters long; it serves to distinguish similar files. The extension can be up to three characters long; it identifies a file type (for example, text files usually end in TXT).

  NOTE: The AI-450 has one major exception to the DOS rules: only five characters are always available to you for naming chromatographic data files. The AI-450 uses the last three characters to identify the detector and to differentiate files created in multiple iterations of a Schedule.

You will often want to work with only one particular type of file. To make this easy, DOS allows you to use wildcards as character variables.

- The character * (commonly called "star") represents any group of characters in a filename or extension. Characters before * will be read, but those following will be ignored. Thus, M*.TXT can represent both MENU.TXT and METHOD.TXT, whereas *.TXT and *Q.TXT represent all text files.
- The character ? represents a single-character position in a filename or extension. Thus, SAL?.* can represent SALT.MET, but not SALINE.MET.

Using Directories To Organize Your Disk

How Directories Are Organized

A fixed disk or a high capacity floppy disk can hold many files. If you keep a large number of files in one place on your disk, it can take longer for DOS to find a particular file. Organizing files into related groups can reduce the time required to locate a file.

DOS organizes files in much the same way that a filing cabinet does. It uses **directories**, which are analogous to file folders, to keep similar files grouped together for easy access and reference. A directory holds information about programs, data files, or other directories.

Directories can be created on different levels. The starting directory of every disk is the **root directory**. DOS automatically creates a root directory on each disk when you format it. The root directory is designated by a backslash immediately following the drive designator (e.g., C:\). The root directory normally contains several files (e.g., AUTOEXEC.BAT, CONFIG.SYS) in addition to the names of additional directories. The root directory of a fixed disk can contain up to 512 files and/or directories (entries).

The next level(s) of directories below the root directory are known as **subdirectories**. Like files, subdirectories have names. Subdirectory names follow the same format as filenames; they can consist of 1 to 8 characters, with no spaces or punctuation.

Subdirectories can contain any number of entries, limited only by the amount of available space on the disk. In Windows, the names of subdirectories appear in the directory tree of the File Manager.

Typically, directories are nested inside one another. The resulting file storage structure resembles an inverted tree, as shown in the figure below. The terms "up" and "down" are often used to describe movement within the directory tree. When you move up, you move from a subdirectory toward the root directory. When you move down, you move away from the root directory toward a subdirectory. The root directory is the "highest" directory.

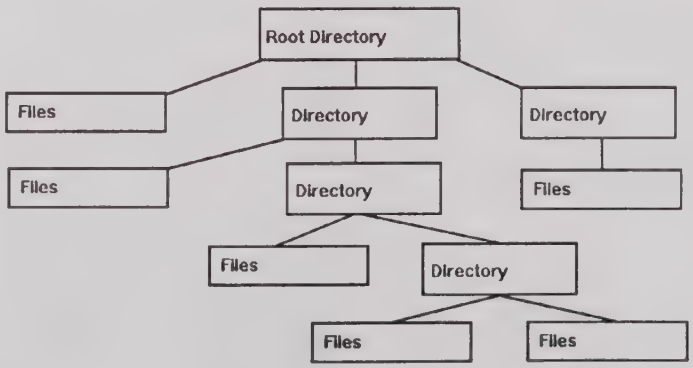


Figure E-2 DOS Directory Structure

Specifying the Pathname for a File

When you want DOS to locate a file, but you do not specify a particular directory, DOS searches only the current directory of your current drive. You can instruct DOS to search for the file in another directory by specifying the drive, the name of the directory, and the filename.

These three items, the drive, the name of the directory, and the filename, can be combined to create a **path** leading to the desired file. This pathname represents the sequence of nested directories that is followed from the root directory to find the specified file. A pathname can contain from 1 to 63 characters and is composed of directory names separated by the backslash (\).

Whenever the backslash is the first character following the drive designator, it indicates the root directory of the disk. Otherwise, the backslash just separates directory names. Whenever you use a filename as the last entry in a path, a backslash is also required between the path and the filename.

For example, to specify a raw data file rawdata.d01, which is stored on the hard disk (drive C:) in the subdirectory ANIONS, which is itself stored in the subdirectory DATA, you would type **C:\DX\DATA\ANIONS\RAWDATA.D01**. Note that the filename is always the last item in the pathname.

The Current Directory

The **current directory** is the one you are currently working in or the one you were working in on a different drive. DOS remembers which directory was current on each of your drives, even if you are not presently accessing any of those drives. Each program running under Windows maintains its own current drive and directory path. You can check a program's current path by using the File Open command; the path will be shown in the pop-up file box. Cancel the command if you do not want to open a file.

When DOS starts, the current directory is determined by the AUTOEXEC.BAT file. You can change directories, using the DOS commands **chdir** or **cd**. Windows makes changing directories even easier: to move up to the next directory level, simply double-click on the name of the desired directory in the current directory file list. To move back down the directory tree, double-click on the desired level in the directory tree. See your Windows manual for additional ways of changing the current directory.

Using Path Strings To Organize Your Data

The AI-450 software allows you to specify a pathname as a part of every filename. This lets you automatically sort data as you generate it. You can also organize Method files and Schedule files.

Before doing this, you must first create the subdirectories. This is easily accomplished by using the Create Directory... command under the File menu in the Windows File Manager. After creating the desired subdirectories, you can have the computer automatically sort data into them by including the appropriate pathname as part of the filename.

EXAMPLE:

Create two subdirectories, one for anions and one for cations, under the directory \DX\DATA. Use these directories to specify the pathname for data files.

1. Display the \DX subdirectory in the Windows Directory Tree Window by double-clicking on **DX** .
2. Change to the data subdirectory by double-clicking on the data directory name in the directory file list. The pathname displayed on the Windows title bar will now be **C:\DX\DATA*.***. The file list will be displayed in the window.
3. Select the Create Directory... command from the File menu in the File Manager. When the file box appears, type in the word **ANIONS** and press [Enter]. When the data subdirectory is redisplayed, notice that the word **ANIONS** now appears in bold letters with brackets in the file list. Repeat the process to create a **CATIONS** subdirectory.

   To ensure that data files are stored in the correct subdirectory, simply include the pathname for the desired directory (e.g., **C:\DX\DATA\ANIONS**) when specifying the Data Filename (see *Method Editor/Data Processing Parameters/Report*). For example, to save a data file under the filename AS8DATA in the anion subdirectory, type the pathname C:\DX\DATA\ANIONS\AS8DATA in the file box and press [Enter].

APPENDIX F - REPORTING SOFTWARE PROBLEMS

As you work with the AI-450 Chromatography Software, you may encounter a situation where the software fails to function correctly. This indicates a software "bug", or problem, and it can be very frustrating.

The purpose of Appendix F is to help you solve the problem as quickly as possible. If you can provide a detailed description of the circumstances, it improves our chances of being able to reproduce, and then eliminate, the bug.

- Can you describe the problem clearly?
- What were you trying to do when the problem occurred? Does the problem occur every time you try to do this? What program were you using?
- Did you notice anything unusual just before the problem occurred? (For instance, did the room lights blink off?)
- What error messages, if any, appeared on your computer screen?

Several SOFTWARE PROBLEM REPORT forms are included at the end of this manual. If you discover a software problem, please fill out one of these forms as completely as possible and mail it to us, or call your local Dionex office for assistance.

If, in the process of reading the manual again to verify that you are doing everything correctly, you find a section of the manual unclear, please write and let us know that, too.

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